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Nanotechnology and nanoscience to manage SARS-CoV-2 variants of concern

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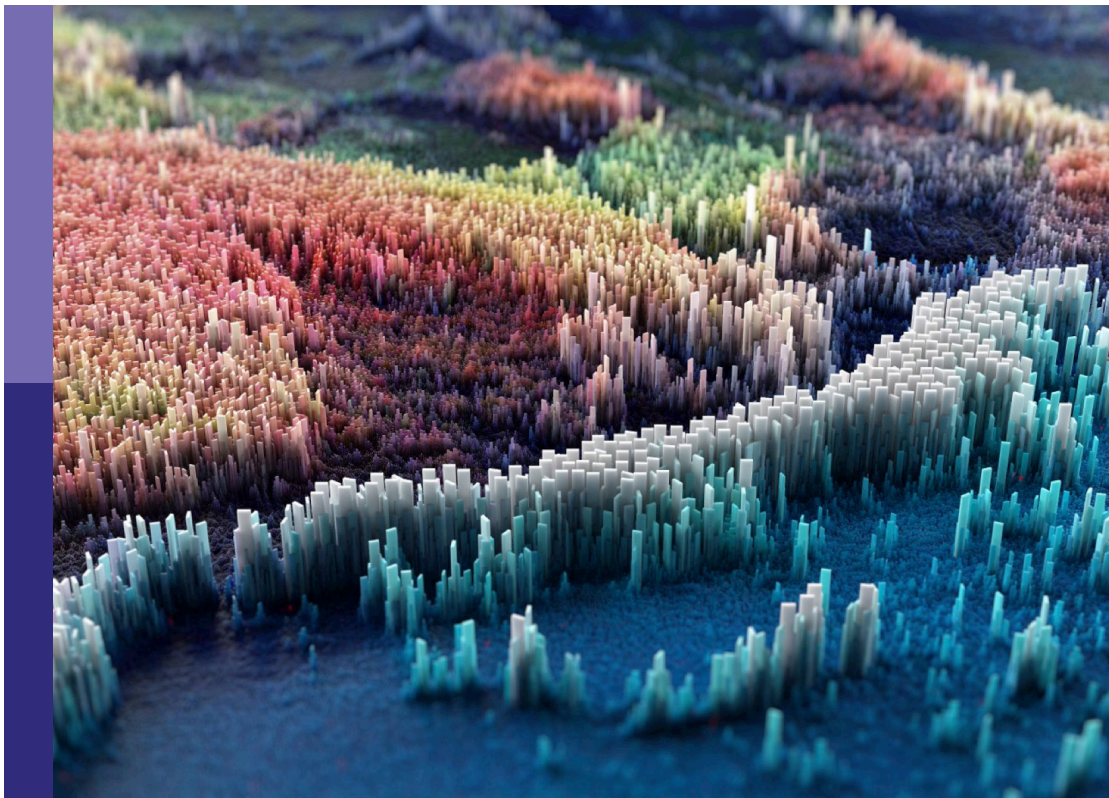

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Nanotechnology and nanoscience to manage SARS-CoV-2 variants of concern

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SARS-CoV-2 variants impact RBD conformational dynamics and ACE2 accessibility

Mariana Valério[1,2†], Luís Borges-Araújo[1,2,3,4†], Manuel N. Melo[1,2*], Diana Lousa[1,2*] and Cláudio M. Soares[1,2*]

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Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has killed over 6 million people and is having a devastating social and economic impact around the world. The rise of new variants of concern (VOCs) represents a difficult challenge due to the loss of vaccine and natural immunity, as well as increased transmissibility. All VOCs contain mutations in the spike glycoprotein, which mediates fusion between the viral and host cell membranes. The spike glycoprotein binds to angiotensin-converting enzyme 2 (ACE2) *via* its receptor binding domain (RBD) initiating the infection process. Attempting to understand the effect of RBD mutations in VOCs, a lot of attention has been given to the RBD-ACE2 interaction. However, this type of analysis ignores more indirect effects, such as the conformational dynamics of the RBD itself. Observing that some mutations occur in residues that are not in direct contact with ACE2, we hypothesized that they could affect the RBD conformational dynamics. To test this, we performed long atomistic (AA) molecular dynamics (MD) simulations to investigate the structural dynamics of *wt* RBD, and that of four VOCs (Alpha, Beta, Delta, and Omicron). Our results show that the *wt* RBD presents two distinct conformations: an "open" conformation where it is free to bind ACE2; and a "closed" conformation, where the RBM ridge blocks the binding surface. The Alpha and Beta variants shift the open/closed equilibrium towards the open conformation by roughly 20%, likely increasing ACE2 binding affinity. Simulations of the Delta and Omicron variants showed extreme results, with the closed conformation being rarely observed. The Delta variant also differed substantially from the other variants, alternating between the open conformation and an alternative "reversed" one, with a significantly changed orientation of the RBM ridge. This alternate conformation could provide a fitness advantage due to increased availability for ACE2 binding, and by aiding antibody escape through epitope occlusion. These results support the hypothesis that VOCs, and particularly the Omicron and Delta variants, impact RBD conformational dynamics in a direction that promotes efficient binding to ACE2 and, in the case of Delta, may assist antibody escape.

KEYWORDS

SARS-CoV-2, receptor binding domain (RBD), variants of concern (VOCs), ridge, MD simulations

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1–3), is a global pandemic with higher mortality than that of seasonal influenza (4). As of July 2022, over 6 million lives had been claimed by this disease (5). Infection by SARS-CoV-2 requires the fusion of viral and host cell membranes, at either the cell surface or the endosomal membrane (6). As for the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome-related coronavirus (MERS-CoV), the SARS-CoV-2 fusion process is mediated by the viral envelope spike (S) glycoprotein (6). Upon viral attachment or uptake, host factors trigger large-scale conformational rearrangements in the S protein, including a refolding step that leads directly to membrane fusion and viral entry (7–12).

The SARS-CoV-2 S protein is composed of a signal peptide located at the N-terminus (residues 1–13) and 2 subunits, S1 (residues 14–685) and S2 (residues 686–1,273) (13). The S1 and S2 subunits are responsible for receptor binding and membrane fusion, respectively (13). The S1 subunit consists of a N-terminal domain (residues 14–305) and a receptor binding domain, or RBD (residues 319–541). In its prefusion state, the S protein exists as a homotrimer and undergoes large conformational changes to control the exposure and accessibility of the RBD. This is done *via* an "up" and "down" mechanism, where the RBD changes from a receptor-accessible "up" conformation to a receptor-inaccessible "down" conformation (14–16).

The RBD is responsible for the interaction of SARS-CoV-2 with host cells *via* binding to the angiotensin-converting enzyme 2 (ACE2) (8, 10, 13, 17), a regulator of the renin-angiotensin system. Binding to ACE2 is one of the first steps in what is considered to be the main mode of SARS-CoV-2 viral entry, hence the importance of the RBD positional change from "down" to "up" (14, 18, 19).

A lot of attention has been given to the SARS-CoV-2 RBD—ACE2 complex due to both its mechanistic implications (20–25) and pharmaceutical potential (26–32). However, not much attention has been given to the dynamics of the RBD by itself. The RBD core structure when bound to ACE2 (**Figure 1A**) consists of a twisted five stranded antiparallel β sheet (β1, β2, β3, β4 and β7), with short connecting helices and loops (33). While most of the S protein surface is densely glycosylated, shielding it from host defense mechanisms, the RBD itself contains only a single glycosylation site (34), N343, which is located relatively distant from the ACE2-RBD interface (**Supplementary Figure S1B**).

This core β sheet structure is further stabilized by 3 disulfide bonds. Between the core β4 and β7 strands (residues 438–506), there is an extended region containing 2 short β strands (β5 and β6), the alpha 4 and alpha 5 helices and loops. This region is the receptor-binding motif (RBM), which contains most of the residues that are responsible for interacting with ACE2 (14, 33). When complexed with ACE2, the RBM folds into a concave surface, that accommodates the N-terminal α-helix of ACE2, with a ridge (residues 471–491) on one side, formed by a disulfide-bridge-stabilized loop (Cys480–Cys488). It is in this surface that several RBM residues establish specific and non-specific interactions with ACE2 residues (33).

From the available experimental structural data the core β-sheet structure is very stable, but the RBM seems to be quite dynamic and not as structurally defined, unless bound to other proteins, like ACE2 (17, 33, 35–37) or antibody fragments (38–44). Molecular dynamics (MD) simulation studies have also mostly focused on RBD complexed with these proteins, and while there are MD simulation studies of the free RBD, they either focus on short simulations (45–47) or do not explore the RBM dynamics in detail (34, 45, 48–50). As such, not much is known about the conformational

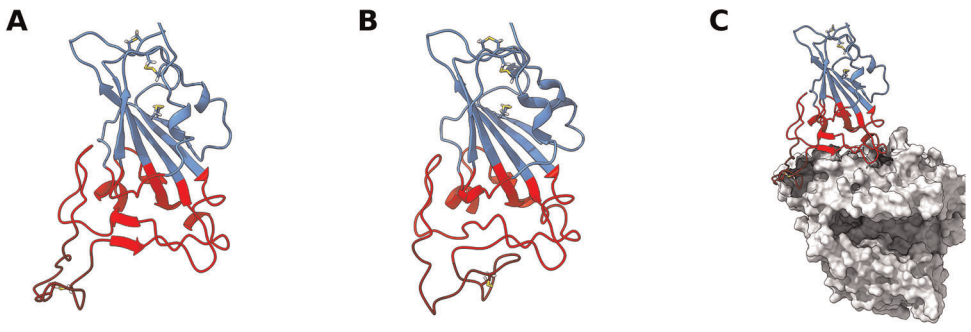

FIGURE 1
SARS-CoV-2 receptor binding domain (RBD) structure. Structure of *wt* RBD in the open (**A**) and closed (**B**) conformations. Snapshots obtained from the AA MD simulations. Disulfide bonds are represented in yellow sticks. Structure of *wt* RBD bound to ACE2 is also shown (**C**). The RBM region is colored red and the ridge in dark red, with the rest of the protein being colored in blue. ACE2 is in grey.

dynamics of this motif when unbound. This is relevant because the conformational dynamics of the SARS-CoV-2 RBD and RBM might not only play an important role in receptor recognition and binding, but also provide important information for the development of newer improved pharmaceuticals.

Recently, a significant number of naturally occurring mutations to the SARS-CoV-2 S protein have also been reported (51–54). Many of these mutations have been identified in the RBD, some of which have given rise to the dominant viral variant in certain regions due to their significant fitness advantage (51–54). Many of these RBD mutations are thought to increase fitness by increasing the binding affinity for ACE2 or by escaping neutralization by anti-SARS-CoV-2 monoclonal antibodies (55). Still, the impact of these mutations on the structural dynamics of the RBD and the RBM have not yet been investigated.

In this work, we use atomistic (AA) molecular dynamics (MD) simulation methods to investigate the structural dynamics of the SARS-CoV-2 RBD, and that of four naturally occurring variants of concern (VOCs): variant B.1.1.7, or Alpha (53) (N501Y); variant B.1.351, or Beta (51) (K417N, E484K and N501Y); variant B.1.617.2, or Delta (52) (L452R, T478K); and variant B.1.1.529, or Omicron (56, 57) (G339D, S371l, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y and Y505H). Our results show that the RBM dynamics of the *wt* RBD are such that it is not always in a conformation competent for ACE2 binding (**Figure 1**). Conversely, all variants, particularly Delta and Omicron, stabilize binding-competent configurations which could increase ACE2 binding efficiency. Besides impacting binding, the large conformational space visited by the variants may also hinder antibody recognition of the RBM region, thus providing a fitness advantage by facilitating antibody escape.

Methods

Molecular dynamics simulations

All atomistic simulations were performed with the GROMACS 2020.3 (58, 59) package and modelled using the Amber14sb forcefield (60), alongside the TIP3P water model (61). The initial *wt* RBD structure was obtained from PDB ID: 6M0J (33), which corresponds to an ACE2 bound conformation of RBD; ACE2 was excluded from this structure. The different RBD variants were generated by mutating the appropriate residues in the *wt* RBD using PyMOL (62).

It is worth noting that glycosylations were not included in our simulation systems. Despite most of the S protein surface being densely glycosylated, the RBD itself contains only a single glycosylation site far from the RBM region (34), where the dynamics reported in this work are observed. Additionally, while it has been reported that other neighboring glycosylation sites can effectively shield the RBD, this glycan shield is paired with its down-to-up conformational change in the complete S-protein: when the RBD is down the glycan shield camouflages the RBD and RBM, however, when the RBD is up it emerges from the glycan shield and presents a fully accessible RBM (34). It is in this up state, when the RBM is fully accessible to the solvent and glycans no longer play a relevant role, that the dynamics we observe may play a role in modulating binding to ACE2. Additionally, accounting for glycans would inevitably introduce degrees of freedom that would complicate sampling. Given that we do not expect glycans to play a relevant role in RBM dynamics, we opted for a reductionist approach by simulating the RBD without glycosylations.

Simulations were performed on each RBD protein structure in water. Each structure was inserted in a truncated dodecahedron box filled with water molecules (considering a minimum distance of 1.2 nm between protein and box walls). The total charge of the system was neutralized with the required number of Na^+ ions, with additional Na^+ and Cl^- ions added to the solution to reach an ionic strength of 0.1 M.

The system was energy-minimized using the steepest descent method for a maximum of 50,000 steps with position restraints on the heteroatom positions by restraining them to the crystallographic coordinates using a force constant of 1,000 kJ/mol in the X, Y and Z positions. Before performing the production runs, an initialization process was carried out in 5 stages of 100 ps each. Initially, all heavy-atoms were restrained using a force constant of 1,000 kJ/mol/nm, and at the final stage only the Cα atoms were position-restrained using the same force constant. In the first stage, the Berendsen thermostat (63) was used to initialize and maintain the simulation at 300 K, using a temperature coupling constant of 0.01 ps, without pressure control. The second stage continued to use the Berendsen thermostat but now with a coupling constant of 0.1 ps. The third stage kept the same temperature control, but introduced isotropic pressure coupling with the Berendsen barostat (63), with a coupling constant of 5.0 ps. The fourth stage changed the thermostat to V-rescale (64), with a temperature coupling constant of 0.1 ps, and the barostat to Parrinello-Rahman (65), with a pressure coupling constant of 5.0 ps. The fifth stage is equal to the fourth stage, but position restraints are only applied on Cα atoms. For production simulations, conditions were the same as for the fifth stage, but without any restraints. In all cases, 2 fs integration steps were used. Long-range electrostatic interactions were treated with the PME (66, 67) scheme, using a grid spacing of 0.12 nm, with cubic interpolation. The neighbor list was updated every twenty steps with a Verlet cutoff with a 0.8 nm radius. All bonds were constrained using the LINCS algorithm (68).

Simulations of each system were performed for at least 7 μs over 5 replicates (the *wt* was simulated for 15 μs, and the Alpha,

Beta, Delta and Omicron variants for 7 μs each). The first 3 μs of simulation were considered as equilibration time and the remaining frames were used for analysis. Visualization and rendering of simulation snapshots was performed with the molecular graphics viewers VMD (69), PyMOL (62) and UCSF Chimera (70).

Principal component analysis

PCA is a standard dimensionality reduction method that we apply here to the (3N-6)-dimensional space of possible RBD conformations (in our case, N being the number of RBD residues). PCA consists of a linear transformation that changes a set of possibly correlated dimensions into a set of linearly uncorrelated, mutually orthogonal ones, called principal components (PCs). The first PC can be defined as the direction that accounts for as much of the variance in the data as possible, with each successive PC accounting for as much of the remaining variance as possible. Reduction of data dimensionality is achieved by retaining only a few of the first PCs—which represent the strongest correlations in the data, in our case, the most important conformational motions—, thus sacrificing some information for simplicity. Discussions of the mathematical and computational backgrounds can be found elsewhere (71–74).

In this work, PCA was applied to sets of conformational coordinates obtained from MD simulations. Prior to PCA, each conformation was translationally and rotationally fitted to the RBD core Cα carbons of the *wt* crystal structure (hence the −6 in the dimensionality). PCs were determined using MDAnalysis (75), from the entire pool of simulation trajectories, considering only the coordinates of the RBD's Cα carbons. The dimensionality was reduced to the 2 most representative PCs, preserving a large part of the variance. RBD structures for each simulation frame, for each variant, could then be projected as points in this two-dimensional space, enabling a simplified visual representation of the conformation space explored by the RBD in each case.

The probability density function for each trajectory projection was estimated using a gaussian kernel estimator (73, 76) implemented in LandscapeTools' *get_density* software as described elsewhere (73, 77). This procedure defines a probability density function $P(r)$, with the values of $P(r)$ being stored for the position of each data point and for the nodes of a two-dimensional uniform grid, with a mesh size of 0.5 Å. These values were used to define an energy surface, calculated as (73):

$$E(r) = -k_B T \ln\left(\frac{P(r)}{P_{max}}\right) \quad (1)$$

Where P_{max} is the maximum of the probability density function, $P(r)$. The energy surface landscapes were analyzed by determining the energy minima and respective basins. The basins were defined as the set of all conformations whose steepest descent path along the energy surface leads to a particular minimum (73, 78, 79). Here, the steepest descent paths for each grid cell were computed, with each conformation inheriting the path of its corresponding grid cell. Landscape regions with $E > 6$ k_BT were discarded, resulting in the final set of basins for each data set.

Residue interaction network analysis

Residue interaction networks (RINs) are graph representations of protein structures, where the nodes represent amino acid residues, and the edges represent interactions between residues. Pairwise residue interactions were analyzed for the 5,000 lowest energy conformations obtained for the most populated open, closed and reversed conformation basins of the energy surface landscapes of each RBD variant, using RIP-MD (80). Several types of interactions between AAs were probed: Cα contacts, hydrogen bonds, salt bridges, disulfide bonds, cation-π, π-π, Arg-Arg, Coulomb and van der Waals. The parameters defining each interaction, as well as their mathematical formulation can be found elsewhere (80). Once the interactions were determined, the interaction networks were visualized using Cytoscape (81).

Results and discussion

Our aim was to study the conformational dynamics of the SARS-CoV-2 RBD, as well as that of several other SARS-CoV-2 VOCs in solution. To this effect, we simulated the *wt*, Alpha, Beta, Delta and Omicron variants of the SARS-CoV-2 RBD. The Gamma variant was not studied due to its similarity to the Beta variant: in the RBD both variants share the E484K and N501Y mutations; the single difference is the K417N mutation in the Beta variant vs. K417T in Gamma (82, 83). In either case, K417 is mutated to a residue with a polar uncharged side chain, which should impact the interaction network similarly. As such, the conformational dynamics specific to the RBD and RBM are expected to be similar.

Wt RBD presents two distinct RBM conformations in aqueous solution

Visual inspection of the trajectories obtained in the simulation of *wt* RBD in water revealed that large dynamic

conformational changes occur in the RBM region (Figure 2A; Supplementary Video S1). The dynamics observed appear to show an opening and closing of the ACE2 binding surface of the RBM. To better characterize these conformational dynamics, we performed principal component analysis (PCA) on the coordinates recovered from these simulations, reducing them to 2 principal components; this 2D configuration space sampling was expressed as free energy landscapes (Figure 2).

For *wt* RBD, we observe two deep basin clusters (Figure 2A), as well as several other lesser populated basins. Closer analysis of the RBD conformations that make up each basin shows that *wt* basins 1 and 3 correspond to conformations close to the ACE2-bound one determined by x-ray crystallography (33) (Figures 1A, 2A). We named these "open" configurations. In contrast, the second basin cluster (basins 0 and 2) was made up by conformations quite distinct from the open ones. In these basins, the loop that makes up the RBM is twisted and collapsed over the region that binds ACE2, effectively hiding it from the solvent (Figures 1B, 2A). We named these conformations "closed". Further analysis of the PCA results reveals that the *wt* RBD is in a closed state for more than half of the simulation time (~55.5%, Supplementary Table S1). Given that in these conformations the RBM closes on itself, hiding the ACE2 binding surface, we can speculate that the RBD would be unable to effectively bind to ACE2 and initiate an ACE2-dependent infection process. Moreover, the open and closed states were visited reversibly (Supplementary Figure S2), indicating that our simulations were not kinetically trapped in either basin. The open and closed RBD conformations reported here should not be confused with the "up" and "down" S protein conformational states which control the exposure of the RBD in the context of the S protein homotrimer. The open/closed dynamics likely act as an additional RBM exposure control, which would be particularly important for RBDs in the "up"

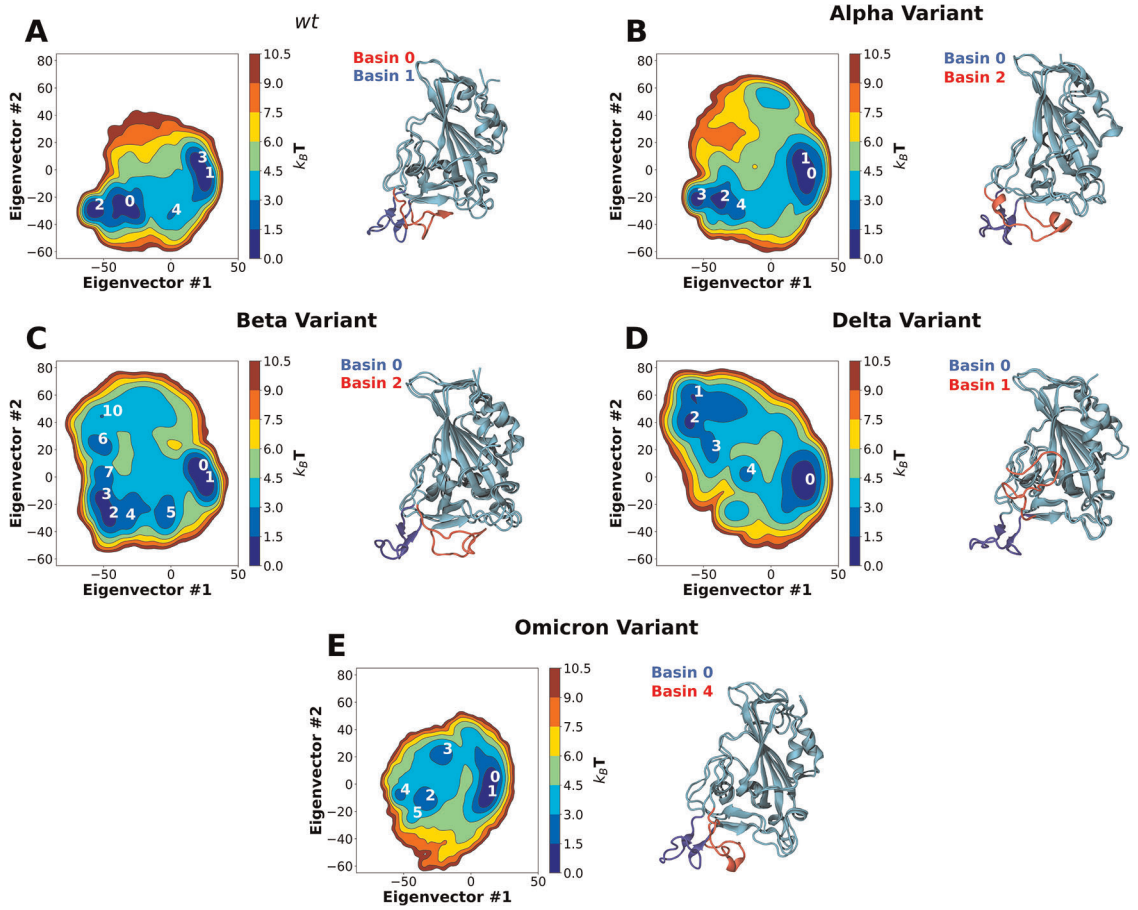

FIGURE 2
Two-dimension principal component analysis (PCA) of SARS-CoV-2 RBD conformational dynamics in water. Plots of the first two principal components determined from the Cα backbone of the *wt* RBD (**A**) as well as the Alpha (**B**), Beta (**C**), Delta (**D**) and Omicron (**E**) variants. Basins with $k_BT < 3$ are numbered in each figure. Snapshots of the lowest energy structures for selected open and closed basins are also shown. The ridge regions of the open and closed snapshots are colored in blue and red, respectively.

S protein conformational state where they are fully exposed to the solvent.

Residue interaction network (RIN) analysis was performed for the 5,000 lowest energy structures of basins 1 (open) and 0 (closed). From the identified interactions, we selected those that were present in over 50% of the simulation frames (**Supplementary Figure S4**). We also only considered interactions that are established by RBM residues, or those in their immediate vicinity. These RINs were then used to probe the different intramolecular interactions established in each of the conformations.

In the open conformation, the RBD ridge is stabilized by a triple π-stacking interaction between residues Y489–F456–Y473 and a hydrogen bond between Y489–Y473. Additionally, two hydrogen bonds are established between residues Y453 and E493, which help stabilize the formation of a small β-sheet (**Figure 3A**).

In the closed conformation, however, the π-stacking interactions are broken, and new interactions with RBD core residues are formed in their place. F456 forms a stable π-stacking with Y421, Y489 forms a transient π-stacking interaction with F486 and Y473 forms a hydrogen bond with the backbone of Y451. Moreover, E484 forms a salt bridge with R403, that is found in the RBD core, and a hydrogen bond with K417 (**Figure 3F**). This hydrogen bond does not show up in the RIN, as K417 can establish a bond with each of the two glutamate oxygens, each with ∼40% prevalence (each thus below our 50% selection cutoff). These two interactions, together with the formation of three hydrogen bonds (C480–S494–G482–Q493) are responsible for the closing of the ridge and consequent shielding of the ACE2 binding surface. The importance of the E484–R403 and E484–K417 interactions for the closing of the loop was confirmed by simulating the E484K and K417N mutants. Either of these single mutations were enough to completely deplete the closed conformation (**Supplementary Figures S3A,B** for E484K and K417N, respectively). This shows that both these interactions are crucial for the stabilization of the *wt* closed state. Still, several other transient hydrogen bonds, formed between residues L492, G493 and S494 of strand β6, and T478, C480, N481, G482 and E484 of the RBM ridge, assist in stabilizing the structure.

The closed conformation does not seem to substantially impact the RBD's secondary structure (**Supplementary Figure S9**). The largest impact appears to be limited to residues 473–474 and 488–489, that in the open state display a slight β-sheet character. However, upon closing, this β-sheet character disappears. This effect comes from residues 473 and

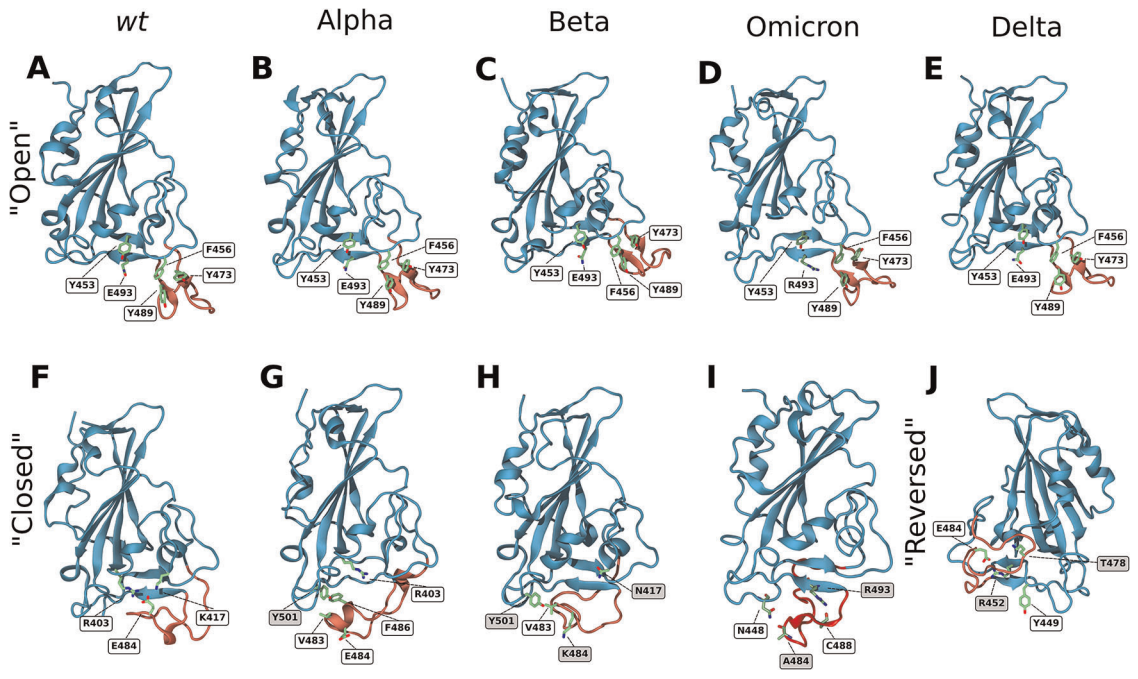

FIGURE 3
Closeup snapshots of SARS-CoV-2 RBD intramolecular interactions that stabilize the various conformations. Snapshots from AA MD simulations showcasing crucial intramolecular interactions responsible for stabilizing the open, closed, and reversed conformations for the *wt* (**A**,**F**), Alpha (**B**, **G**), Beta (**C**,**H**), Omicron (**D**,**I**) and Delta (**E**,**J**) RBD variants. The ridge region of the RBD is colored in red and residues of interest in green. Text labels indicate relevant residues, with shaded labels indicating mutations relative to *wt*. All figures are rotated 180° relative to **Figures 1**, **2**, apart from snapshot J.

489 no longer participating in the triple π-stacking that was likely stabilizing this region.

Apart from impacting ACE2 accessibility, the closing of the RBM ridge also decreases the solvent accessible surface area (SASA) of the RBD by slightly over 3% (**Supplementary Table S2**).

Although other studies have noted the high flexibility in the RBM region of the RBD (45–47, 84), this is, as far as we know, the first report of this hinge mechanism that can effectively hide the ACE2 binding surface of the RBD from binding partners, which could only be observed through the analysis of μs-long MD simulations. While it is likely that induced fit interactions might assist in opening a closed conformation for binding to ACE2, it is safe to assume that the closed conformation will have its binding to ACE2 substantially hindered when compared to an open conformation. Other studies have also observed RBM concealment mechanisms in the context of the spike protein (84). It has been shown that "down" state RBDs can conceal their RBM by interacting with the neighboring RBDs, in a temperature dependent manner. This further showcases the tendency of the RBM to conceal its hydrophobic surface, either by closing in on itself (as observed in the present study) or by interacting with neighbouring RBDs [as observed by Rath et al. (84)].

SARS-CoV-2 alpha and beta variants impact RBM conformational dynamics and exposure

The first SARS-CoV-2 variant of concern to be identified was first detected in the UK. It is often referred to as B.1.1.7 or Alpha variant and has only one mutation in the RBD region—N501Y. A second variant emerged soon after in South Africa, independently of B.1.1.7, referred to as B.1.351 or Beta variant. In the RBD region, this variant shares the N501Y mutation with the Alpha variant and includes two others: K417N and E484K (53).

In line with what was observed for the *wt* RBD, MD simulations of the RBDs from the Alpha and Beta variants also showed the prevalence of two sets of RBM conformations, corresponding to open and closed conformations (**Supplementary Videos S2, S3**). PCA analysis of the Alpha variant trajectory shows two deep basin clusters (**Figure 2B**), basins 0 and 1, and basins 2 and 3, which correspond to open and closed conformations respectively. However, unlike the *wt* variant, the Alpha variant remains most of the simulation time in an open conformation (~72.64%, **Supplementary Table S1**). The Beta variant (**Figure 2C**) also has two deep basin clusters (basins 0 and 1, and basins 2 and 3), corresponding to open and closed conformations, respectively. Like the Alpha variant, Beta remains in an open conformation for substantially longer time than the *wt* (~69%, **Supplementary Table S1**). In both cases, and as for *wt*, our simulations were able to reversibly visit both states (**Supplementary Figure S2**).

Both Alpha and Beta variants shift the open/closed equilibrium towards more open conformations by roughly 20%. An opening ΔΔG was calculated from the ratio between the time spent in the open and closed states, where the time spent in each individual open and closed basin was added together (**Supplementary Table S1**). The equilibrium shift led to a decrease in the opening ΔΔG from 0.55 ± 0.17 kJ/mol, in the case of *wt* RBD, to -2.44 ± 0.22 and -2.09 ± 0.14 kJ/mol, for the Alpha and Beta variants, respectively. As mentioned previously, it is likely that only the open conformations are fully available to bind to ACE2, meaning that these mutations substantially increase the accessibility of RBD to ACE2, and probably impact ACE2-RBD binding.

By analyzing the intramolecular residue interactions for both variants, we observe that the interactions which stabilize the open conformation in the *wt* RBD are conserved in both Alpha and Beta variants, namely the triple π-stacking between residues Y489–F456–Y473, as well as the hydrogen bond between Y489 and Y473. An additional hydrogen bond between Q493 and Y453 assists in stabilizing the β6 strand (**Figures 3B,C**).

Interestingly, in both the open and closed conformations of the Alpha variant, the interactions established by residue Y501 (Alpha's only mutation in the RBD) that were previously present in the *wt* variant are maintained in the Alpha variant (two hydrogen bonds established through the residue backbones: Q458–Y501 and Y501–Q506). However, the main interactions that stabilize the closed conformations differ between the Alpha variant and *wt* (although some transient hydrogen bonds between strand β6 and the RBM ridge do remain). Instead of the E484–R403 salt bridge seen for *wt*, in the Alpha variant the closed conformation is promoted by the formation of hydrophobic interactions between the mutated Y501, V483 and F486 (**Figure 3G**). This arrangement hinders the establishment of the E484–R403 salt-bridge (as can be seen in **Supplementary Video S2**) while being itself less stable than the open conformations. This is the likely cause for the decrease in percentage of closed state observed for Alpha. Progression to the E484–R403 salt-bridge may also be prevented in part by the establishment of a short α-helix, discussed ahead.

In the Beta variant, the closed conformation is notably impacted by both the E484K and the N501Y mutations. The E484K mutation prevents the formation of the E484–R403 salt bridge that was crucial for the stability of the closed conformation in the *wt* protein. However, unlike the single E484K mutant (**Supplementary Figure S3**), the Beta variant can still reach a closed conformation. This is because it can establish the same hydrophobic interaction between Y501 and V483 as the Alpha variant (**Figure 3H**). This closed state is

also stabilized by the same transient hydrogen bonds between strand β6 and the RBM ridge seen in the *wt* and Alpha variants.

Concerning the secondary structure, there are no substantial differences between the Alpha or Beta open states and the *wt* open state (**Supplementary Figure S9**). However, upon closing, both Alpha and Delta form a small α-helix between residues 475 and 490, for roughly 30% of the simulation time. This helical character might be relevant for the Alpha variant, as it assists in facing the E484 sidechain away from R403 (**Figures 3G,H**), hindering the formation of the salt-bridge. Additionally, the Alpha variant also shows some helicity in residues 482–489, which likely arises from contacts between residues in this helix and the mutated N501Y.

Curiously, while the Alpha variant also shows a considerable decrease in SASA upon closing (∼5%), the Beta variant shows no substantial change.

Overall, these results showcase a possible alternative mechanism for how the Alpha and Beta variants might facilitate viral entry into the host cells. By shifting the open/closed equilibrium towards the ACE2-accessible open conformation, both variants are facilitating ACE2–RBD binding, which will inevitably lead to an increase in binding affinity and enhanced receptor-dependent infection.

SARS-CoV-2 delta variant shows conformational dynamics distinct from the other variants

During the second half of 2021 the global dominant SARS-CoV-2 variant was B.1.617.2 (or Delta) (52). It contains two mutations in the RBD region: L452R and T478K. Like the *wt*, Alpha and Beta variants, MD simulations of the Delta RBD show the prevalence of two sets of RBM conformations, one of which corresponds to the *wt* open conformation (**Supplementary Video S4**) and is stabilized by the same interactions observed for the three other variants (**Figure 3E**). However, unlike those variants, MD simulations of the Delta RBD do not show the occurrence of a closed conformation at all. Instead, an alternative open conformation is present, which we refer to as "reversed". PCA analysis of the Delta variant trajectory, shows two deep basins, 0 and 2 in **Figure 2D**, which correspond to the open and reversed conformations, respectively. Similarly to the other variants, simulations were able to reversibly visit the two states (**Supplementary Figure S2**).

The reversed conformation showcases the incredible flexibility of the RBM region, which not only opens and closes over the ACE2 binding surface of the RBD but acts as a two-way hinge that leans to the side of the RBD. This alternative conformation might also prove significant advantages over the *wt* open state: RBD-targeting antibodies are known to bind *via* recognition of the RBM ridge region (22, 85); the reversed state putatively hides this region from antibody recognition, while still providing an open ACE2 binding surface for infection.

A hydrogen bond between the mutated R452 on strand β5 and Y449 appears to be one of the main driving forces folding the Delta variant's ridge region backwards. This interaction destabilizes the β5 strand and enables the ridge to move up and interact with the core. Transient interactions between ridge residues G476, S477 as well as the mutated K478 with residues R346, F347 and N354 of strand β1 stabilize the contact between the ridge loop and the RBD core, keeping it locked in place (**Figure 3J**).

Regarding the secondary structure, much like the other variants, the Delta open conformation is very similar to that of the *wt* (**Supplementary Table S3**). However, as expected, the reversed conformation shows substantial differences. In this state, the two small beta strands formed by residues 473–474 and 488–489, present in the open conformation, are completely lost. Additionally, the beta-sheet formed by strands β5 and β6 becomes less prevalent, likely due to the L452R mutation (one of the β5 strand residues that destabilizes the β-sheet by establishing a new interaction with Y449). Curiously, like in the Alpha and Beta variants, there is also a significant alpha helical character between residues 490 and 475.

As for the closed conformations of the *wt*, Alpha and Beta variants, the Delta reversed conformation also leads to a decrease in SASA (∼3%). Unlike the closed conformations, however, this alternative open conformation still presents a fully accessible ACE2 binding surface.

SARS-CoV-2 omicron variant further improves RBM accessibility compared to alpha and beta

Towards the end of 2021 a new VOC—B.1.1.529 or Omicron—overtook Delta as the dominant variant in most world regions (86). The Omicron variant is highly distinct from other VOCs (87), containing 15 mutations in the RBD region (G339D, S371l, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y and Y505H), 10 of which are concentrated in the RBM (N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y and Y505H). Some of these mutations are also observed, or are similar to those, in the Alpha, Beta and Delta variants: K417N, T478K, E484A and N501Y.

Unlike the other variants, MD simulations of the Omicron RBD do not show a clear prevalence of two distinct sets of RBM conformations (**Figure 2E**; **Supplementary Video S5**). While PCA analysis shows a deep basin cluster corresponding to the *wt* open conformation, there are only shallow basins corresponding to the closed conformation. No alternative reversed conformation is observed. The open conformation accounted for almost the entire set of configurations sampled

during the simulations of the in Omicron variant (~95%, Supplementary Table S1), substantially larger than that of the *wt*, Alpha or Beta variants. The simulations were able to reversibly visit either state (Supplementary Figure S2). When compared to the *wt* variant, Omicron resulted in a 50% shift in the open/closed equilibrium towards more open conformations, with an opening $\Delta\Delta G$ of -8.38 ± 0.5 kJ/mol.

The triple π-stacking interactions and hydrogen bonds that stabilize the open conformation in the Omicron variant are common to those present in the *wt*, Alpha, Beta and Delta variants (Figure 3D). However, the closed conformation is quite distinct from the one seen with the other variants (Figure 3I). Only two transient hydrogen bonds—one between the sidechain amide of N448 and the backbone of A484, and the other between the sidechain of R493 and the backbone of C488—are in place to stabilize the closed conformation. This contrasts with the stronger interactions present in the *wt*, Alpha and Beta closed conformations. The *wt* closed conformation was stabilized by the formation of two salt bridges between K417, E484 and R403. In Omicron, E484 is mutated to an alanine preventing these two interactions. In the Alpha and Beta variants, instead of these salt bridges, several hydrophobic interactions between Y501, V483 and F486 promoted the closing of the loop. The Omicron Y501N mutation disrupts this hydrophobic core. It is clear that several mutations to the Omicron RBM actively hinder the closing of the loop while promoting the open conformation, which ultimately facilitates ACE2-RBD binding.

No substantial differences in secondary structure between the Omicron and *wt* variants in either the open or closed states were observed (Supplementary Figure S9).

It is worth noting that the Omicron variant has recently been classified into five different lineages based on their mutations, BA.1, BA.2, BA.3 (56, 88, 89), BA.4 and BA.5 (90). The BA.1 lineage was used in this work. Regarding the RBD region, all five lineages have 11 mutations in common (G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y and Y505H). BA.1 has three specific mutations (S371l, G446S and G496S), BA.2 has four (S371F, T376A, D405N and R408S), BA.3 has a combination of BA.1 and BA.2 mutations (G446S, S371F and D405N) and BA.4 and BA.5 have several mutations in common with BA.2 (S371F, T376A, D405N and R408S), plus two others (L452R and F486V).

According to our simulations, the mutations most associated with the open/closing dynamics of the RBD ridge are, for the most part, common to the BA.1, BA.2, BA.3, BA.4 and BA.5 lineages (E484A, Q493R, N501Y, and K417N). The only exception to this is the Q493R mutation in the BA.4 and BA.5 lineages, which does not occur and instead the *wt* Q493 residue is maintained. Additionally, the mutations specific to the BA.2 and BA.3 lineages are relatively far from the RBM loop region and as such are unlikely to play a role in this mechanism (Supplementary Figures S16B,C). Two of the mutations specific to BA.1 are present in this region (G446S and G496S, Supplementary Figure S16A). However, these mutations did not play an obvious role in the RBD ridge dynamics, and we speculate that reverting these residues back to glycines would not impact these dynamics significantly. Taking all of this into consideration, we expect that the RBD dynamics of the other Omicron lineages to be fairly similar to that of BA.1.

Impact of SARS-CoV-2 variants on ACE2 binding affinity

To find experimental basis for our results, we compiled ACE2-RBD binding kinetics data from recent studies (91–102) (Supplementary Table S3). These results were obtained by surface plasmon resonance (SPR) and biolayer interferometry (BLI) and encompass data regarding both the *wt* and studied variants. Additionally, we compiled results obtained for just the RBD as well as for the entire S protein. While the binding kinetics values recovered from these studies are not fully consistent with each other, likely due to differences in particular experimental setups, they are mostly in the same range, and appear to follow similar trends. Regarding the equilibrium dissociation constant (K_d), all variants have an increased binding affinity when compared to the *wt*. However, with the currently available data, it is hard to distinguish between the efficiency of the several variants, with the Alpha and Beta variants showing a slightly better affinity than Delta.

To get more information, we analyzed both the association (k_{on}) and dissociation rate constants (k_{off}). k_{off} reflects the lifetime of the protein-protein complex and as such, the strength of the interaction. We observe a consistent decrease in k_{off} for the variants in comparison to the *wt*. The Alpha and Beta variants stand out from Delta and Omicron in this regard, with substantially lower k_{off} values. These results hint at the VOCs interacting more strongly with ACE2 than the *wt*, with the Alpha and Beta complexes being substantially more stable than those of Delta and Omicron. Several other MD studies have studied the impact of these mutations on ACE2-RBD contacts, binding affinity and binding modes, showcasing how the substantially altered ACE2-RBD interaction of the Alpha and Beta variants might be outperforming that of the *wt* variant (103–108). The Delta variant does not contain mutations to the RBD ACE2 binding surface and, as such, the interactions established are not substantially different from those of *wt*. This is reflected in a k_{off} that is closer to, if still lower than, that of the *wt*.

The variants also substantially impact k_{on}. This rate constant reflects the efficiency with which protein–protein collisions lead to a bound state. While a couple of studies show no significant impact (91, 95), most show that the variants lead to a substantial increase in k_{on}, reflecting an increase in RBD accessibility to ACE2 (92–94, 96, 101). We

propose that this can be explained by the significant changes in RBM conformational dynamics that we have here described, where mutations lead to a decrease in prevalence of the closed state, favoring binding. As such, our results point to an alternative mechanism for enhancing RBD-ACE2 binding, not by directly strengthening ACE2-RBD interactions, but rather by boosting, *via* modulation of ridge dynamics, the ACE2 binding competence.

Conclusion

In this work we performed AA MD simulations of the SARS-CoV-2 RBD, as well as that of the Alpha, Beta, Delta and Omicron VOCs, to characterize the impact of the mutations on RBD conformational dynamics in solution. Our results show that the *wt* RBD adopts two distinct conformations in equilibrium: an open conformation where the RBD is free to bind ACE2; and a closed conformation, where the RBM ridge blocks the ACE2 binding surface and likely hinders binding to ACE2. We characterized the two states and showed that they originate from specific intramolecular interactions between residues of the RBM ridge and those of the surface that binds ACE2. As far as we know, this is the first report of this "hinge-like" mechanism, which can effectively shield the surface of RBD binding ACE2 from the solvent and binding partners. This mechanism is yet to be seen in experimentally solved RBD structures, which have thus far struggled to fully resolve the unbound RBM region (14, 25, 109). The RBM is found unresolved in most structures due to the large flexibility of the region, and those that are fully resolved are often structures of RBD complexed with either ACE2 (17, 33, 35–37), antibodies (38–44) or itself by dimerizing *via* the surface binding ACE2 (110, 111).

The four variants tested in this work, significantly impacted the open/closed equilibrium we observed for *wt* RBD. Both Alpha and Beta variants shifted the equilibrium towards more open conformations by roughly 20%. In Omicron the open conformation accounted for 96% of simulation time while the Delta variant did not show the presence of a closed conformation at all. This shift towards more open conformations likely enhances ACE2 binding affinity by increasing accessibility to the RBM and facilitating binding. Several experimental binding studies have shown that these variants lead to a substantial increase in ACE2-RBD binding association rate constant, reflecting an increased ACE2 accessibility, in agreement with our findings.

Additionally, the Delta variant showed an alternative open conformation, distinct from that of the other variants. This alternative conformation keeps the ACE2 binding surface open and accessible for binding, but significantly alters the conformation of the RBM ridge. This state presents a substantially altered ridge region, which bends backwards towards the RBD core, shielding some of it from exposure. We hypothesize that this may provide a fitness advantage by aiding in antibody escape, since many RBD-targeting antibodies bind to the RBM ridge region (39, 85, 112, 113). These RBD-targeting antibodies are also more sensitive to viral evolution than antibodies that bind other regions of the RBD (114). In the alternative open conformation, the ridge may be not as easily recognized, while the ACE2 binding surface remains unobstructed for infection. The substantially different conformational dynamics of the RBM region between the variants, correlates well with the hypothesis proposed by Quaglia et al. that mutations are enriched at intrinsically disordered regions of the SARS-CoV-2 proteome and that they may contribute towards immune evasion (115).

These results show that the mutations found in the four VOCs studied impact RBD conformational dynamics in a direction that promotes efficient binding to ACE2 and (in the case of the Delta variant) antibody escape, an effect which has thus far been disregarded. In this context, our findings can also help explain some of the antibody-evading characteristics of the emergent Omicron variant.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for this study in accordance with the local legislation and institutional requirements.

Author contributions

MV and LB-A contributed equally to this work. DL and CS designed the simulation setup. DL prepared the systems' topologies and CS performed the simulations. MV, LB-A, MM, DL and CS designed the analysis and MV and LB-A performed them. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmedt.2022.1009451/full#supplementary-material.

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Exploring nanoselenium to tackle mutated SARS-CoV-2 for efficient COVID-19 management

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Despite ongoing public health measures and increasing vaccination rates, deaths and disease severity caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its new emergent variants continue to threaten the health of people around the world. Therefore, there is an urgent need to develop novel strategies for research, diagnosis, treatment, and government policies to combat the variant strains of SARS-CoV-2. Since the state-of-the-art COVID-19 pandemic, the role of selenium in dealing with COVID-19 disease has been widely discussed due to its importance as an essential micronutrient. This review aims at providing all antiviral activities of nanoselenium (Nano-Se) ever explored using different methods in the literature. We systematically summarize the studied antiviral activities of Nano-Se required to project it as an efficient antiviral system as a function of shape, size, and synthesis method. The outcomes of this article not only introduce Nano-Se to the scientific community but also motivate scholars to adopt Nano-Se to tackle any serious virus such as mutated SARS-CoV-2 to achieve an effective antiviral activity in a desired manner.

KEYWORDS

COVID-19 infection, SARS-CoV-2, nanoselenium, antiviral, antimicrobial

1 Introduction

The transmission of coronaviruses (CoV) in animals has a long history (Ruiz-Aravena et al., 2022). However, the earliest pathogenic transmission from animal to human was detected as a severe acute respiratory syndrome (SARS) in Guangdong province, China, in 2002, and after a decade, one more pathogenic coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), was identified in Middle Eastern countries (Cui et al., 2019; Dhama et al., 2020; Shereen et al., 2020). The recent COVID-19 outbreak originated in the city of Wuhan, China, and rapidly spread all over the world and has become a new global public health crisis (Cui et al., 2019; Zhu et al., 2020). After the declaration of novel coronavirus disease as a pandemic by the World Health Organization

(WHO), researchers in academics and pharmaceuticals have been increasingly engaged in the search for effective antiviral drugs and therapeutic treatment options. The emergence of SARS-CoV-2 variants through mutations to their genetic code is a growing concern for public health. The Centers for Disease Prevention and Control (CDC), United States, recently revised the classification of variants and included another class of variants named as variant being monitored (VBM) in addition to three preceding classes of SARS-CoV-2 variants, that is, variant of concern (VOC), variant of interest (VOI), and variant of high consequence (VOHC), to prioritize global monitoring, selective and rapid diagnosis, research to explore pathogenesis, and treatments of higher efficacy (Centers for Disease Prevention and Control, 2022).

The structural proteins of SARS-CoV-2 include four different structural proteins, such as spike (S) glycoprotein, membrane (M) protein, small envelope glycoprotein (E), and nucleocapsid (N) protein, and are responsible for viral replication and propagation (Schoeman and Fielding, 2019; Yadav et al., 2021). Like other viruses, SARS-CoV-2 is susceptible to genetic evolution while transmitting from one host person to others through mutations over the time (Harvey et al., 2021). These changes have little or no impact; however, some mutations could alter its pathogenic or transmission potential of the virus and can amplify disease severity (Young et al., 2020). Throughout the pandemic, several variants of SARS-CoV-2 have been identified around the world. So far, five variants, namely, B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and B.1.1.529 (Omicron), have been listed as variants of concern (World Health Organization, 2021). Despite the remarkable progresses of vaccine and drug development against COVID-19 disease, the emergences of new variant strains of SARS-CoV-2 can not only result in an increased rate of spread, disease severity, and mortality but also threaten to overturn the significant progress made so far in halting the spread of SARS-CoV-2. SARS-CoV-2 variants have raised concerns about resistance to neutralizing by escaping from host antibody responses and have become a stumbling block to ending this pandemic. Therefore, prevention, vaccination adoption, and advancing research are key factors for the management of the COVID-19 pandemic.

Various kinds of nanotechnology-based materials have been studied in biomedical science, including metal NPs, quantum dots (QDs), carbon-based nanosystems, and polymeric nanomaterials (Chenthamara et al., 2019; Aflori, 2021; Singh et al., 2021a; Ma et al., 2021; Dhiman et al., 2022a; Dhiman et al., 2022b; Chaudhary *et al.*, 2022). In the biomedicine field, nanoparticles hold great promise for a variety of applications, including drug developments, diagnostic techniques, drug delivery, gene delivery, nanodrugs, and prostheses and implants (Pelaz et al., 2017; Kaushik, 2019; Malachowski and Hassel, 2020; Nienhaus et al., 2020; Li et al., 2022; Mostafavi et al., 2022; Xie et al., 2022). These applications are revolutionary in the biomedical field and result in new materials, techniques, and devices with more sensitivity, selectivity, and sophistication, which are required for this field (Kaushik et al., 2020; Ahmadivand et al., 2021; Sharma et al., 2021). In the ongoing pandemic, nanotechnology plays an important role in dealing with SARS-CoV-2 and its variants (Paliwal et al., 2020; Gage et al., 2021; Sadique et al., 2021; Varahachalam et al., 2021; Dubey et al., 2022; Kujawska et al., 2022). Unlike many other metals, Se is an essential element in the human body. Therefore, Nano-Se having very low toxicity has better medicinal properties over other metal nanoparticles (Ferro et al., 2021). In the last two decades, biological activities of selenium nanoparticles (Nano-Se) toward various diseases have been extensively studied (Figure 1), which has transformed the Se nanoparticle chemistry as a major branch of study in nanotechnology (Khurana *et al.*; 2019; Shi et al., 2021; Bisht et al., 2022). The capabilities of Se-containing species to act as an antiviral in nature make it notable and potentially useful in the current COVID-19 pandemic (Kieliszek, 2022; Singh et al., 2022). In the case of SARS-CoV-2, Se deficiency is related to increased susceptibility to infections (Khatiwada and Subedi 2021; Majeed et al., 2022). This observation has been observed in many findings from different countries, where the selenium status was associated with cure rates of COVID-19 disease (Zhang D. W et al., 2020; Erol et al., 2021; Majeed et al., 2021). Moreover, studies on other virus-related diseases such as HIV, influenza, and Ebola demonstrated the benefit of Se supplementation (Harthill, 2011; Ivory et al., 2017; Muzembo et al., 2019). A considerable number of research articles and review articles on synthesis, characterization, and the wide range of applications of Se nanoparticles are proof of the growth in this field. This review projects the potential of Se nanoparticles and its decorated systems for antiviral applications systematically. This review article aims to summarize the studied antiviral properties of selenium nanoparticles and other related materials.

2 Origin of selenium

In nature, Se is a common element and exists in organic and inorganic forms. The typical organic forms of Se are selenocysteine and selenomethionine, which are present in the human body, while the inorganic forms include selenite and selenate, which are available in plants (Gupta and Gupta, 2017). Since the discovery of Se in 1817, the role of selenium in the human body has evolved and its deficiency has been shown to correlate with the progression of HIV to AIDS, cardiovascular disease, infertility, Keshan disease, cognitive decline, and impaired mineralization of bones and teeth (Dworkin, 1994; Benstoem et al., 2015; Pieczyńska and Grajeta, 2015; Munguti et al., 2017; Yang et al., 2022). On Earth, Se is unevenly distributed and concentrated in different forms, which leads to variation in Se intake throughout the population of the

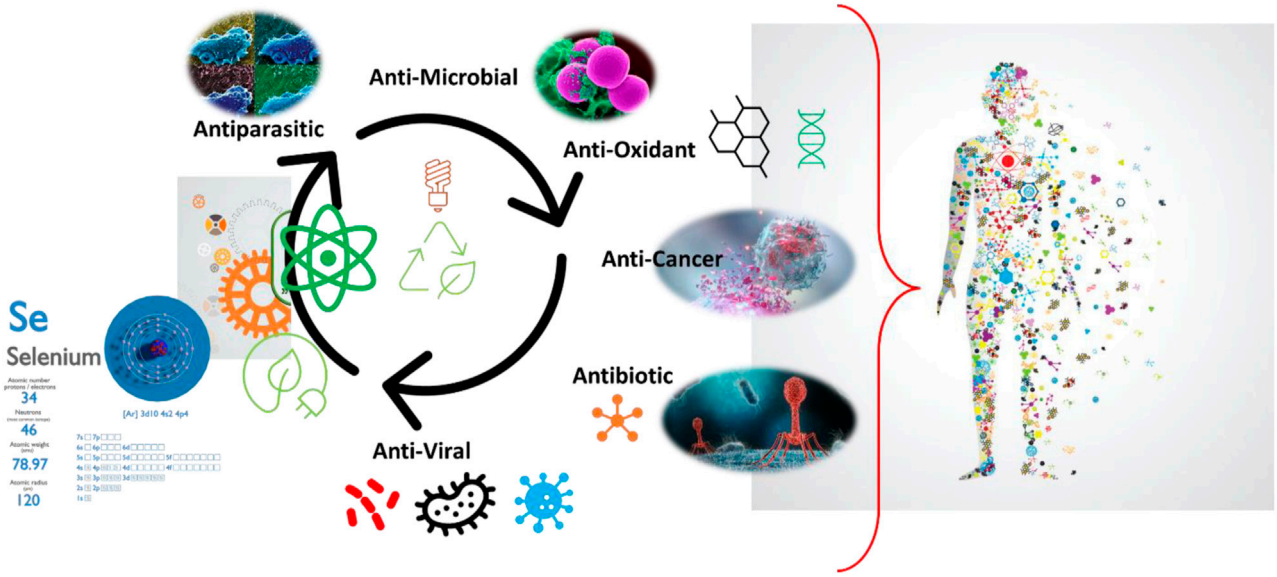

FIGURE 1
Biological activities of selenium nanoparticles (Nano-Se) and related systems.

TABLE 1 Selenium intake for children and adults (Institute of Medicine Food and Nutrition Board, 2000).

Age (year)	Male/female	
	Recommended dietary allowance (ug/day)	Upper tolerable limit (ug/day)
0–0.6	15	45
0.7–1	20	60
1–3	20	90
4–8	30	150
9–13	40	280
14+	55	400
Pregnancy	60	400
Lactation	70	400

world (Kieliszek et al., 2022). It is estimated that the diets of at least one billion people in the world lack sufficient Se for their well-being (Haug et al., 2007). An adequate selenium status is essential for regulating many physiological processes in the human body (**Table 1**).

Se cannot be produced by human cells and must be ingested through food (Kieliszek and Blazejak, 2016). Selenium enters the food chain through plants uptake from the soil. Crops cultivated in soil with a low Se concentration are correlated with Se deficiency in the human population. Selenium biofortification in plant strategies to produce an adequate Se amount in foods can be helpful in overcoming Se deficiency and improving human health. Apart from traditional biofortification methods, researchers continue their efforts to find new advanced Se biofortification methods, including genetic engineering, microbial-based biofortification, and through organic matter recycling (Sarwar et al., 2020). In human diet, the main contributors to Se intake are plant-based products (46.5%) (Hu et al., 2021). Animal-based foods are the second highest source of Se intake, in which fish, meat, meat-based products, milk, dairy products, and eggs are the main dietary sources (Hu et al., 2021). Selenium has vital roles in animals; they must ingest selenium through food, and in biological processes they transform the inorganic Se form to the organic form (Se-Cys). The selenium amount in animal-based foods depends upon the selenium in the plants or food that animals eat and the species of animals (Hu et al., 2021). The incorporation of Se in animal feed can increase the Se content in animal-based products and result in indirect introduction of selenium into the human body (Pavlovic et al., 2018).

3 Clarity on Se toxicity

The first scientific finding on Se as a toxic form was found through multiple experiments conducted at the Wyoming and South Dakota Agricultural Experiment Stations in 1929 (Zwolak, 2020). In these experiments, it was found that crops grown on specific soil caused an alkali disease to livestock (Christophersen

et al., 2013). However, with the confirmation of an essential role as a trace element in the diet of mammals (Kieliszek and Blazejak, 2016), the focus of most research efforts shifted to exploring Se deficiency and has prompted studies on the biological activities of different forms of selenium species (Dhau et al., 2014a; Dhau et al., 2015). The United States Department of Agriculture in 1974 approved inorganic selenium as food additive to animal feeds in the identified regions where selenium was at a subadequate level in soil (Surai et al., 2018). In 1978, scientists recognized selenocysteine moiety as a catalytic site in glutathione peroxidase, later named the "twenty-first" amino acid by Bock et al.(1991) (Schmidt and Simonovic, 2012).

All forms of Se are potentially toxic to living species, with the toxicity dependent upon the chemical nature of Se, dose, the route of administration, age of species, physiological state, and nutrition and dietary interactions (Nogueira et al., 2004; Christophersen et al., 2013; Singh et al., 2021b). Selenium poisoning in humans has been reported in different situations, such as accidental ingestion, industrial mishappenings, and environmental toxicity (Nuttall, 2006). The most serious events of environmental toxicity to humans occurred in China with symptoms including loss of hair and cracked nails (Huang et al., 2013). The lowest observed adverse effect level (LOAEL) for selenium is 1540–1600 μg/day of Se while 5,000 μg/day of Se is the toxic level, which can cause selenosis (Stoffaneller and Morse, 2015; Prabhu and Lei, 2016; Ibrahim et al., 2019). Selenosis is the term used for conditions resulting from chronic selenium toxicity and has symptoms of loss of hair, damaged nails, cardiac complications, and respiratory problems (Stoffaneller and Morse, 2015; Ibrahim et al., 2019).

Se in nanodimensional forms, that is, nanoselenium (Nano-Se), is less toxic than other forms of selenium (organic and inorganic) (Hosnedlova et al., 2018; Bhattacharjee et al., 2019; Bisht et al., 2022). Selenium-based nanomaterials have been investigated for many medical applications, such as diagnosis and imaging, antioxidant, antibacterial and antiviral, and chemotherapy (Sakr et al., 2018; Khurana et al., 2019; Bisht et al., 2022). Nanoselenium has the advantage of selenium in its zero-oxidation state (Se^0), which introduces high bioavailability and lower toxicity (Wang et al., 2007; Shi et al., 2010). Cytotoxicity analysis of Nano-Se and SeO_2 demonstrated that Se nanoparticles were six times less toxic against MCF-7 cells (Forootanfar et al., 2014). In another study, selenium nanoparticles were studied for safety, toxicities, biocompatibility, and antitumor activity in animal models and in addition to the high value of therapeutic effectiveness, no significant value of toxicity was found (Mittal and Banerjee, 2021). To deal with the toxic nature of the selenium species at higher concentrations, Dhau et al. (2014b)explored the polymer matrix as a sustained and controlled release drug device for selenium compounds. Discoveries such as these open new possibilities for the development of new classes of selenium-based drugs.

4 Prospects for Nano-Se to tackle SARS-CoV-2 variants

In general, the development of new drugs is a difficult and lengthy process, which takes at least 10 years on an average to complete the journey from initial discovery to the marketplace (Mohs and Greig, 2017). The ongoing pandemic caused by SARS-CoV-2 and its muted variants has recognized the urgency to discover new antiviral drugs and, with this, the idea of repurposing existing drugs to treat infectious viruses emerged as an attractive strategy (Martinez, 2022). All over the world, various health agencies and research groups have performed various studies such as computational research, clinical trials, and other experiments to study repurposed standard drugs and potential drug candidates to treat COVID-19 symptoms (Wang and Guan, 2021). Jin et al. (2020) investigated a pool of 10,000 molecules including standard drugs, drug molecules in clinical phases, and pharmacologically relevant molecules to identify efficient inhibitors of main protease (M^{pro}), a key enzyme of SARS-CoV-2, using the combination of structure-based drug design, virtual drug screening, and high-throughput screening. Of the results, out of six active compounds against SARS-CoV-2 M^{pro}, ebselen exhibited promising antiviral activity in cell-based assays (Jin et al., 2020). The main protease, M^{pro} activity, is critical for breaking the viral polyprotein into further protein units for virus replication and pathogenesis (Moghadasi et al., 2020). The position of SARS-CoV-2 M^{pro} inhibitor in the prevention of SARS-Cov-2 propagation is shown in Figure 2 (Mengist et al., 2020). Ebselen [2-phenyl-1,2-benzisoselazol-3(2H)-one] is an organoselenium compound, which is most widely studied as a GP_x mimetic and has also been found to possess antiviral properties against a number of viruses such as human immunodeficiency virus type 1 (HIV-1), hepatitis C virus (HCV), influenza A virus, Zika virus, herpes simplex virus type 1 (HSV-1), and coxsackie virus (Wójtowicz et al., 2004; Mukherjee et al., 2014; Singh et al., 2015; Oostwoud et al., 2016; Sharma et al., 2017a; Sands and Back., 2018; Simanjuntak et al., 2018; Zhang J et al., 2020). In addition to ebselen, researchers have been exploring the other selenium compounds for various biological activities (Dhau et al., 2014b; Sharma et al., 2017b; Dhau et al., 2021; Kumar et al., 2022). Amporndanai et al. (2021)investigated the binding modes of ebselen derivatives in M^{pro} through high-resolution co-crystallography and further explored their chemical reactivity using spectroscopic techniques. The authors found that two ebselen derivatives have better inhibitory effectiveness than ebselen against M^{pro} enzyme and SARS-CoV-2 replication. These studies have fueled the discussion and research on the potentials of synthetic forms of selenium to manage SARS-COVID-19 (Kieliszeka and Lipinskib, 2020; Seale et al., 2020; Siesa and Parnham, 2020; Zhang J et al., 2020; He et al., 2021; Khatiwada and Subedi, 2021; Kieliszek, 2022; Pedrosa et al., 2022).

In biological science, the research on Se-containing species was started with the focus on the treatment of oxidative stress, which can be responsible for several diseases (Tinggi, 2008). In

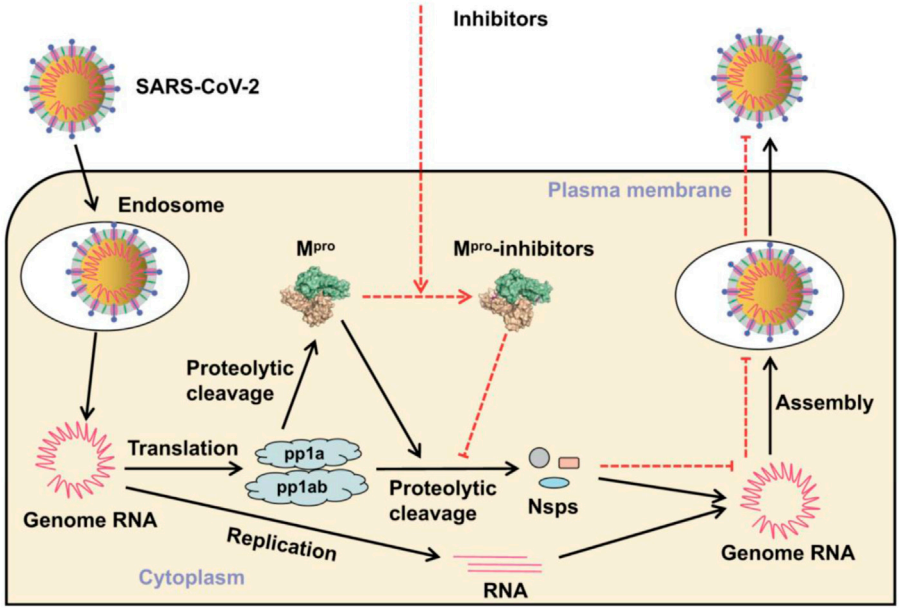

FIGURE 2
Position of the inhibitor in the prevention of SARS-CoV-2 replication (Mengist et al., 2020).

the early studies on SARS-CoV-2, scientific findings concluded that its infection in the respiratory system can activate nuclear factor kappa B (NF-κB), which leads to multiple inflammatory and autoimmune diseases (Hirano and Murkami, 2020; Davies et al., 2021). Selenium is well-established to affect NF-kB activity and contributes to reducing viral-related infection (Hiffler and Rakotoambinina, 2020). Nuclear factor kappa B (NF-κB) transcription factors control several critical physiological processes, including oxidative stress, inflammation, cell growth, apoptosis, immune responses, and the expression of certain viral genes (Lin et al., 2009; Oeckinghaus and Ghosh, 2009). Kumari et al. (2018)tested a combination of curcumin-loaded Se nanoparticles and CD44-targeted doxorubicin-loaded nanoparticles for anticancer activity and found a lower expression of NF-κB. Ge et al. (2021)compared the alleviation of Cd-induced heart inflammation through inhibiting the NF-κB pathway using three different forms of Se, namely, Se-enriched yeast (organic form), sodium selenite (inorganic form), and Nano-Se (prepared through bioreduction), and demonstrated superior results with the nanoform of Se. Researchers have investigated the role of essential metals as micronutrients in surviving the COVID-19 challenge. Micronutrients including zinc (Zn), copper (Cu), iron (Fe), and selenium (Se) are involved in the better management of the immune system functioning against viral infections, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Renata et al., 2022). Zhang D. W. et al. (2020)performed a study including 17 Chinese cities and showed that Se insufficiency was associated with an elevated COVID-19 mortality risk. Selenium supplements have proven health benefits in other viral disease prevention (Zhou et al., 2018).

The trace element Se is known for a regular functioning of the immune system by increasing the production of immunoglobulin G and M antibodies and leading to increased activity of T cells and macrophages in human health. This biological outcome of Se obtained from its presence in more than 25 selenoproteins was identified in humans (Calder et al., 2020; Maltseva et al., 2022). Five glutathione peroxidases (GPx1-4 and GPx6), three thioredoxin reductases (TrxR1-3), and three deiodinases are the major types in the list of 25 selenoproteins (Bates et al., 2000; Moghadaszadeh and Beggs, 2006; Flohé et al., 2022). GPx1 is a cytosolic enzyme present in all tissues, and a decrease in this level causes an increase in the production of reactive oxygen species (ROS), which leads to oxidative stress, activation of NF-κB transcription, and cell apoptosis (Youn et al., 2008; Lubos et al., 2011; Ren et al., 2020).

5 Antiviral properties of Nano-Se

Nano-Se are fascinating species that have been explored by researchers to produce advanced materials for various applications (Chaudhary and Mehta, 2014; Dong et al., 2014; Peng et al., 2015; Kolay et al., 2019; Ferro et al., 2021). Many biological studies were performed using Nano-Se, including

TABLE 2 Nanoselenium particles and their conjugated systems with studied antiviral activity against different viruses.

Se/conjugated system (size), synthesis method	Virus	Studied on	Model	Result	Reference
Nano-Se (10–250 nm), biosynthesized-from actinobacterium	Type-1 dengue virus	Vero cloned cell lines	*In vitro*	Inhibited at 700 ppm	Ramya et al. (2015)
Nano-Se (100 nm), chemically	Hepatitis B virus	Female inbred BALB/c mice	Oral feeding to mice	Increase in Th1 immune response	Mahdavi et al. (2017)
Nano-Se (10–25 nm), chemically	HPAI-H5N1 virus	Animal study on chickens	Diets supplemented with Nano-Se concentrations	Superior results found using 1 mg Nano-Se/kg in combination with 0.1 mg/dose vaccination	Yehia et al. (2022)
Nano-Se (200 nm), chemically	H1N1	MDCK cells	*In vitro*	Survival rate increased to 82.5% using 0.25 mM Nano-Se	Liu et al. (2022)
SeS_2 nanoparticles (500 nm), precipitation method	A/H1N1 and Betacoronavirus 1	MDCK and HCT-8	*In vitro*	More than 99% inhibition	Długosz et al. (2022)
Nano-Se (200 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 67%	Wang et al. (2020)
Nano-Se@β-thujaplicin (80 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 88%	Wang et al. (2020)
Nano-Se (200 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 60%	Li et al. (2017)
Nano-Se@OTV (100 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 93%	Li et al. (2017)
Nano-Se (-), chemically	Enterovirus 71	Human astrocyte U251 cells	*In vitro*	Cell viability: 38.6%	Zhong et al. (2019)
Nano-Se@OTV (10 nm), chemically	Enterovirus 71 virus	Human astrocyte U251 cells	*In vitro*	Cell viability: 83.2%	Zhong et al. (2019)
Nano-Se (142 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 41.4%	Lin et al. (2017)
Nano-Se@ZNV (82 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 72.7%	Lin et al. (2017)
Nano-Se (200 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 58.87%	Li et al. (2018)
Nano-Se@AM (70 nm), chemically	H1N1	MDCK cells	*In vitro*	Cell viability: 70.26%	Li et al. (2018)
Nano-Se (200 nm), chemically<	H1N1	MDCK cells	*In vitro* and *in vivo*	Cell viability: 65.2%	Lin et al. (2018)
Nano-Se@RBV (<100 nm), chemically	H1N1	MDCK cells	*In vitro* and *in vivo*	Cell viability: 80.6%	Lin et al. (2018)
Nano-Se (200 nm), chemically	H1N1	MDCK cells	*In vitro* and *in vivo*	Cell viability: 65%	Li et al. (2019)
Nano-Se@ARB (70 nm), chemically	H1N1	MDCK cells	*In vitro* and *in vivo*	Cell viability: 86%	Li et al. (2019)
Chitosan–Nano-Se	Porcine reproductive and respiratory syndrome virus	African green monkey kidney epithelial cell line	*In vitro*	Cell viability: IC_{50} = 99.35 μM	Shao et al. (2022)

Nano-Se, selenium nanoparticles; MDCK, Madin–Darby canine kidney; H1N1, influenza virus; (HPAI)-H5N1, highly pathogenic avian influenza; OTV, oseltamivir; ZNV, zanamivir; AM, amantadine; RBV, ribavirin; and ARB, arbidol.

antiviral, antimicrobial, anti-oxidant, anticancer, antidiabetic, and antiparasitic (Khurana et al., 2019; Shi et al., 2021; Bisht et al., 2022). Several antiviral investigations were performed using Nano-Se and their conjugates (summarized in Table 2). The progress in the development of nanoselenium species and related systems as antiviral active agents is discussed in the remaining section.

Ramya et al. (2015) prepared Se nanoparticles through aerobic biogenic synthesis from actinobacteria, having an average particle size of 10–250 nm. They investigated applications of these nanoparticles for antibiofilm, antioxidant, wound healing, cytotoxic, and antiviral activities. The antiviral activity of biosynthesized Se nanoparticles was tested against the type-1 dengue virus using different concentrations

(100–1000 ppm) of Se nanoparticles. The biosynthesized Se nanoparticles showed maximum inhibition of type-1 dengue viral growth at 700 *ppm* concentration.

Selenium is a vital micronutrient in the human body that plays a dominant role in many processes such as the maintenance of cellular redox homeostasis in nearly all tissues, thyroid hormone metabolism, modulation of the immune system by antiviral properties, and enhancement of humoral immunity response (Huang et al., 2012). Mahdavi and co-workers explored the effect of Se nanoparticles on inducing a Th1cytokine pattern after hepatitis B surface antigen (HBsAg) vaccination in animal models. In this study, authors measured lymphocyte proliferation, interferon gamma, interleukin 4, and total antibody by enzyme-linked immunosorbent assay (ELISA) (Mahdavi et al., 2017). The results clearly demonstrated that oral administration of synthetic Se nanoparticles to mice helped in increasing Th1 immune response and caused the production of cytokines, which play a crucial role in the immune response against viral infection and other diseases.

Nano-Se of 200 nm was prepared from combining Na_2SeO_3 (0.1 M) and vitamin C and verified for inhibition of influenza (H1N1) virus (Liu et al., 2022). Influenza A (H1N1) virus infection in humans not only leads to respiratory diseases but also occasionally causes severe lethal pneumonia and nervous system damage. As influenza viruses become less susceptible to antiviral drugs, therefore, in the search of new potential drugs with different modes of action, Liu *et al.* investigated selenium nanoparticles for an antiviral study against H1N-infected Madin–Darby canine kidney (MDCK) cells. In this investigation, a change in morphology of MDCK cells infected by H1N1 was observed using the microscopic technique and shrinkage of cytoplasm, loss of cell integrity, and lower cell numbers were found (Figure 3A). The cell survival rate after treatment with H1N1 virus was studied using different concentrations of selenium nanoparticles by MTT assay. Results are shown in Figure 3, recommending that selenium nanoparticles (Nano-Se) with 0.25 mM concentration increased the survival rate up to 82.5% compared to the control samples. In a mechanistic investigation, GPx1 activity was determined using different concentrations of Nano-Se and we found that improving the level of GPx1 plays a role in the inhibition of H1N1 influenza virus-induced apoptosis.

Yehia et al. (2022) utilized a combination of Nano-Se and inactivated highly pathogenic avian influenza for studying the improvement in chicken immunity. Two methods of Nano-Se supplementation were used: first in diets with Nano-Se concentrations (0.25–1 mg/kg) and then vaccinated and in the second method, 0.5 ml of the vaccine used with 0.02–0.1 mg/dose of Nano-Se. The group of chickens having selenium supplementation of 1 mg Nano-Se/kg showed a considerably higher antibody titer at four and following weeks in the post-vaccination study. In both groups, Nano-Se supplementation via diet or vaccine dose resulted in a lower viral shedding and milder symptoms of inflammation in the lung, trachea, spleen, and liver. Among all chickens, the best vaccine efficacy was observed in a group of chickens fed with 1 mg Nano-Se/kg in combination with 0.1 mg/dose vaccination.

Długosz et al. (2022) (Figure 4) have reported the preparation of Se sulfide nanoparticles using the precipitation method, in which aqueous solutions of sodium sulfide and selenium chloride in the presence of cinnamon, curcumin, and cayenne pepper extracts were extracted using natural deep eutectic solvents (NDESs). This innovative method produced selenium sulfide nanoparticles stabilized with extract of species in natural deep eutectic solvents and has increased bioactivity toward microorganisms. Natural deep eutectic solvents were based on the different combinations of citric acid, propanediol, betaine, proline, lactic acid–glucose, and betaine, and the authors found that using these solvents, the highest content of active ingredients from the spices could be extracted. The average size of selenium sulfide nanoparticles observed by X-ray diffraction analysis was about 500 nm. The antiviral activity of as prepared selenium sulfide nanoparticles was tested against human influenza virus A/H1N1 and Betacoronavirus 1. Using the nanoform of selenium sulfide in curcuma extract, the antiviral results showed a 5.5 log reduction in TCID50 virus titer against A/H1N1, which means more than 99.99% virus inactivation and in the case of HCoV-OC43 virus, and the decrease in the virus infectivity was 99.93% with the virus titer reduction logTCID50 of 3.2.

The other developments in the search for new antiviral drugs have been strategically based upon the conjugation of Nano-Se with standard antiviral drugs. Most of these studies present in the literature are based on the antiviral activity against H1N1 virus. H1N1 is a subtype of influenza A virus and is a common human pathogenic agent, which causes serious respiratory illness (Lin et al., 2017). In 2009, the pandemic caused by the influenza virus started in Mexico, and the most recent SARS-CoV-2 pandemic has severely affected humankind, causing numerous deaths, and both remain a serious problem in public health (Wang et al., 2020). Both these viruses may circulate simultaneously, which can cause more serious respiratory diseases. Bao et al. (2021) developed systematic animal models (ferrets and mice) of co-infection of H1N1 with SARS-CoV-2 for studying the consequences and pathogenesis of sequential infections. This study showed that both viruses together can enhance the condition of pneumonia in ferrets and mice and concluded that an effective prevention strategy from these viruses is concurrent vaccinations against these viruses. These types of findings may be helpful in developing systems against multivirus infections that lead to improvement in public health.

Selenium nanoparticles (Nano-Se) were conjugated with zanamivir (ZNV) and explored for antiviral properties (Lin et al., 2017). Zanamivir is an antiviral drug used as an inhibitor for effective treatment against influenza A and influenza B viruses. The antiviral activities of ZNV, Nano-Se, and ZNV-decorated selenium nanoparticles (Nano-Se@ZNV)

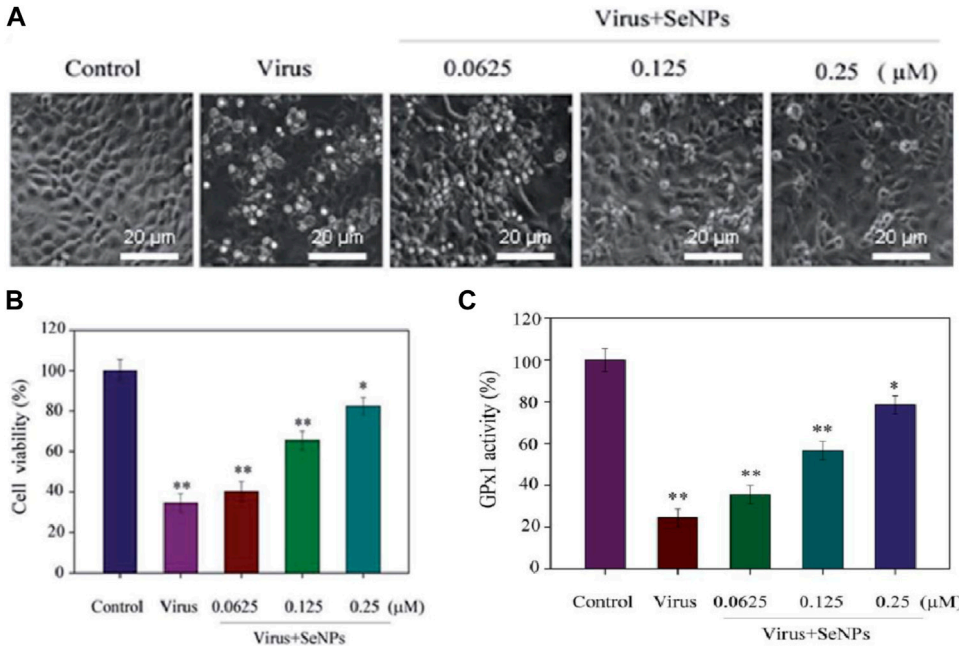

FIGURE 3
(A) Phase contrast microscopic study on the morphology of H1N1-infected MDCK cells using different concentrations of selenium nanoparticles. **(B)** Antiviral activity through cell viability percentage using the MTT assay. **(C)** GPx activity percentage using different concentrations of Nano-Se (Liu et al., 2022).

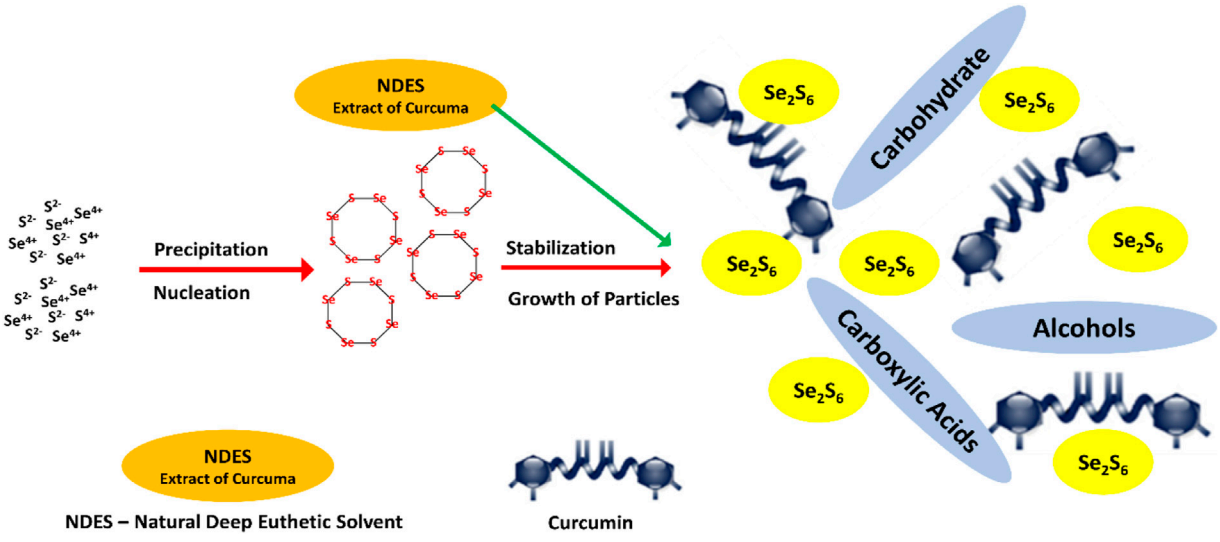

FIGURE 4
Synthesis of Se sulfide nanoparticles in NDES extract of curcuma (Długosz et al., 2022).

were studied using the MTT assay. The results of this study indicated that ZNV-decorated Nano-Se have a greater antiviral activity compared to the Nano-Se and ZNV alone. Madin–Darby canine kidney cell viability after treatment with Nano-Se@ZNV reached 72.7% from 62.2% cells treated with ZNV (Figure 5A). Furthermore, the RNA level of H1N1 after treatment with different groups (ZNV, Nano-Se, and Nano-Se@ZNV) was determined, and it indicated that the cells treated with Nano-

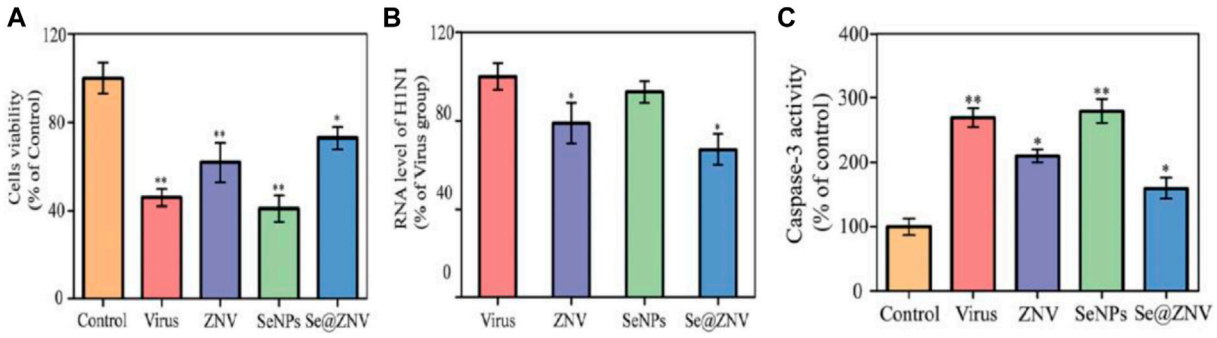

FIGURE 5
(A) Antiviral activity through cell viability percentage. **(B)** Percentage of the RNA level of H1N1. **(C)** Caspase-3 activity of infected MDCK cells (Lin et. al., 2017).

Se@ZNV had a lower RNA level of H1N1 virus than those with the standard antiviral drug alone (Figure 5B). H1N1 influenza virus induced apoptosis of MDCK cells, in which caspase-3 plays a pivotal role. Therefore, the authors studied caspase-3 activity of MDCK cells infected by H1N1 virus in different groups and found 273% and 161% in cases without treatment and Nano-Se@ZNV treatment, respectively; results are shown in Figure 5C. This substantial drop demonstrates the antiviral activity of Nano-Se@ZNV.

In another study, Nano-Se were functionalized with antiinfluenza drug oseltamivir (OTV) and their synergic effect on antiviral properties against H1N1 virus was explored (Li et al., 2017). OTV-modified Nano-Se (Nano-Se@OTV) as globular nanocomposites were prepared in a spherical shape of size 100 nm. Transmission electron microscopy (TEM) and size distribution results showed a reduced size of Nano-Se@OTV compared to Nano-Se (Figures 6A–D). *In vitro* antiviral activity results indicated that Nano-Se@OTV are superior for inactivation of H1N1 virus compared to the Nano-Se and OTV alone. Using TEM characterization, the authors investigated the effect of Nano-Se@OTV on the H1N1 morphology (Figures 6E,F). Using Nano-Se@OTV, another study was conducted on enterovirus 71 (EV71) in the human astrocytoma cell model (Zhong et al., 2019). Enterovirus 71 (EV71) is a single-stranded RNA virus and has been associated with hand, foot, and mouth disease (HFMD) in young children. It was found that Nano-Se@OTV were able to penetrate into human astrocyte U251 cells and suppressed EV71 proliferation. Further, in a detailed mechanism study, the authors found that protection of EV71-infected U251 cells from apoptosis was initiated by the mitochondrial pathway, thereby reducing the generation of reactive oxygen species.

Li et al. (2018)used amantadine (AM) to decorate the surface of Nano-Se (Nano-Se@AM) and reported that Nano-Se@AM has the ability to inhibit H1N1 influenza virus to infect MDCK cells through suppression of the neuraminidase activity. In MTT assay, MDCK cells have cell viability of 32.34, 53.23, 58.87, and 79.26% for virus alone, virus with AM, virus with Nano-Se, and virus with Nano-Se@AM, respectively (as shown in Figure 7A). Again, similar to previous studies on the combination of Nano-Se and standard drugs, Nano-Se@AM showed superior performance toward antiviral properties. The neuraminidase enzymatic activity study was used to investigate the antiinfluenza mechanism and identified that Nano-Se@AM bind tightly to the neuraminidase protein, resulting in the difficulty of the attachment of H1N1 virus to MDCK cells as the responsible factor for the antiinfluenza action of Nano-Se@AM. Moreover, in the mechanistic study, the ROS generation was recorded using dichlorodihydrofluorescein (DCF) assay. In the control, the infection of MDCK cells with H1N1 influenza virus without any treatment increased the reactive oxygen species generation up to 420%. Using Nano-Se@AM, the ROS generation level was reduced to 130% (shown in Figure 7B). This study showed that ROS was a potential novel target for antiviral drug development.

Ribavirin (RBV)-loaded selenium nanoparticles (Nano-Se@RBV) with a diameter of 65–100 nm were studied for inhibition of H1N1 influenza virus infection (Lin et al., 2018). Nano-Se@RBV increased MDCK cell viability after H1N1 infection and reduced viral titers in the culture supernatant by resisting the caspase-3 activation pathway. The authors further extended the antiviral activity study in *in vivo* models using H1N1 influenza-infected mice. It was observed that levels of lung injury and DNA damage were diminished in *in vivo* samples using Nano-Se@RBV compared to untreated groups. The result on restriction of activation of the caspase-3 signaling pathway coincidence between *in vitro* and *in vivo* studies. H&E and tunnel staining were used for demonstrating the efficiency of Nano-Se@RBV for prevention of DNA and lung damage during H1N1 infection (Figure 8).

In another study, arbidol (ARB)-loaded selenium nanoparticles (Nano-Se@ARB) showed effectiveness in reduction of the virus by following the pathway of the

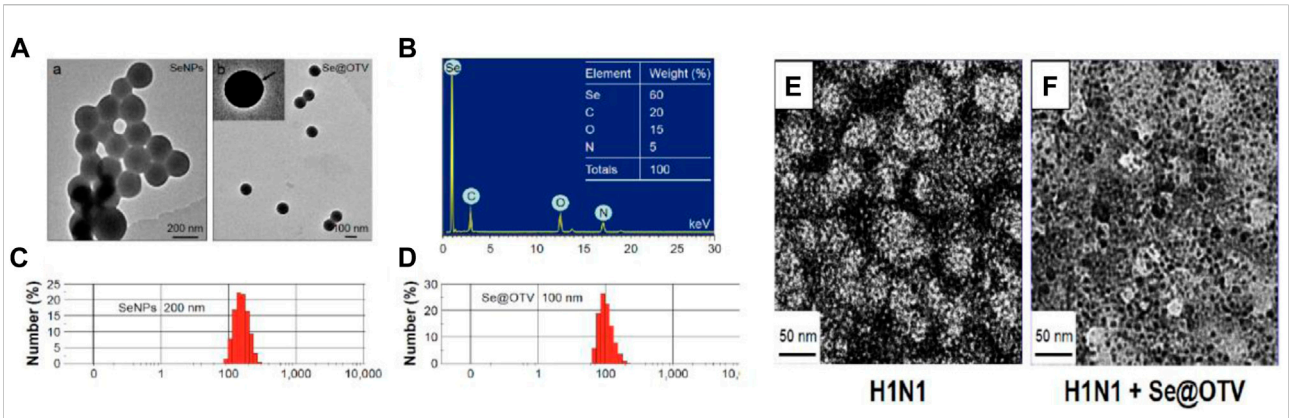

FIGURE 6
(A) Transmission electron microscopy images of Nano-Se and Nano-Se@OTV. **(B)** Energy-dispersive X-ray analysis of Nano-Se@OTV. **(C,D)** Size distribution of Nano-Se and Nano-Se@OTV. **(E)** Control samples of H1N1. **(F)** Treated sample of H1N1 with Nano-Se@OTV (Li et al., 2017).

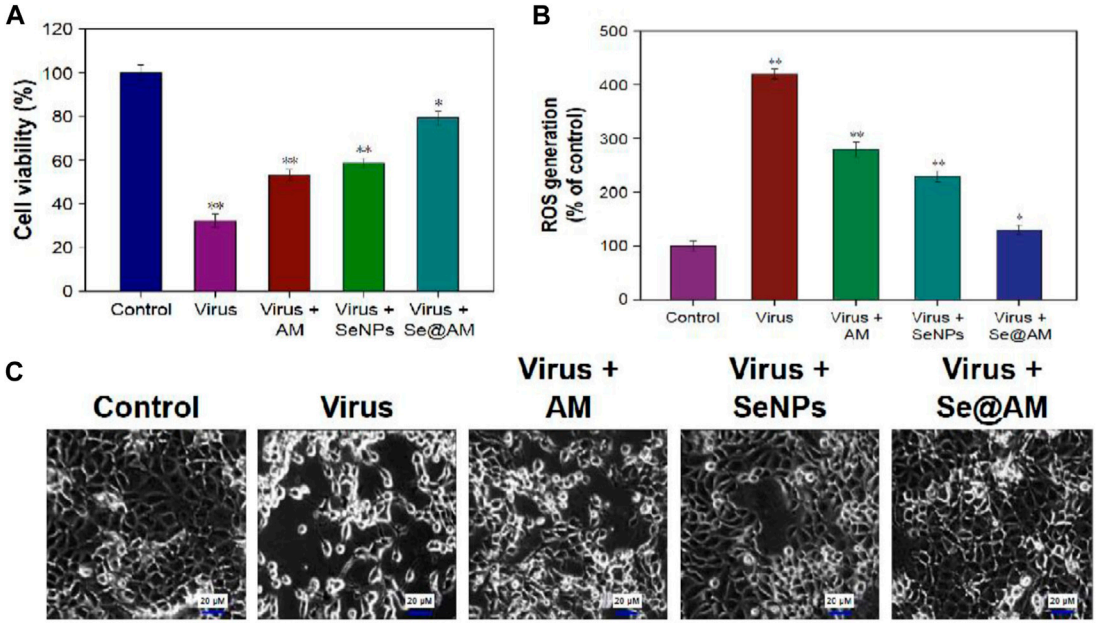

FIGURE 7
(A) Antiviral activity through cell viability percentage using the MTT assay. **(B)** ROS generation using different treatments detected by DCF fluorescence intensity. **(C)** Phase contrast microscopy images showing morphological changes in H1N1-infected MDCK cells (Li et al., 2018).

inhibition of hemagglutinin and neuraminidase (Li et al., 2019). Also, suppression of virus-induced oxidative stress of H1N1 infected MDCK cells through a signaling cascade involving p53, and AKT played an important role in antiviral activity. Novel functionalized selenium nanoparticles by β-thujaplicin (Nano-Se@TP) were evaluated for antiinfluenza activity (Wang et al., 2020). Effects and mechanisms of Nano-Se@TP in inhibiting H1N1 from infecting MDCK cells were studied. In a detailed mechanistic study, authors demonstrated that Nano-Se@TP inhibited caspase-3-mediated apoptosis by inhibition of ROS-mediated AKT and p53 signaling pathways.

Shao et al. (2022)used chitosan, a nontoxic, easily biodegradable, and biocompatible linear polysaccharide to coat Nano-Se. The prepared chitosan-coated Nano-Se investigated for antiviral performance against porcine reproductive and respiratory syndrome virus (PRRSV) infected African green monkey kidney cell line MARC-145. The results revealed that the increase in apoptosis rates induced by PRRSV infection was considerably reduced by chitosan–Nano-Se through suppression of oxidative

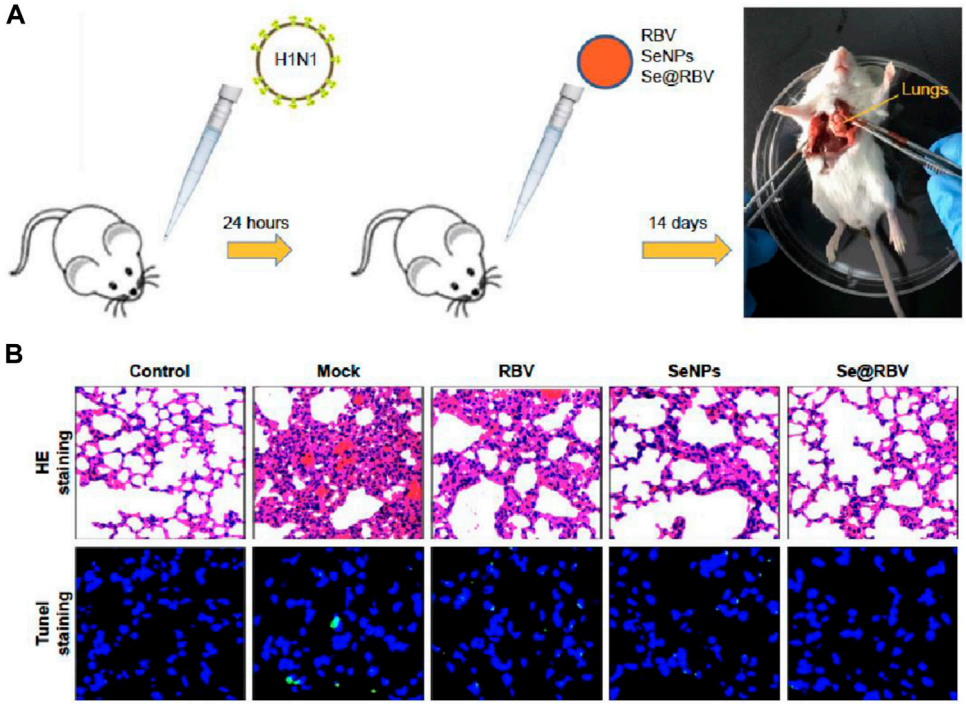

FIGURE 8
(A) Mice infected by H1N1 virus was treated with physiological saline (Mock), RBV, Nano-Se, or Nano-Se@RBV. **(B)** HE staining and tunnel staining (Lin et al., 2018).

stress by the combination of increased GSH-Px activity, promoted GSH production, and inhibited H_2O_2 synthesis. In a cell viability experiment, the IC_{50} value of chitosan–Nano-Se for African green monkey kidney cell line MARC-145 was recorded at 99.35 μM (Figure 9).

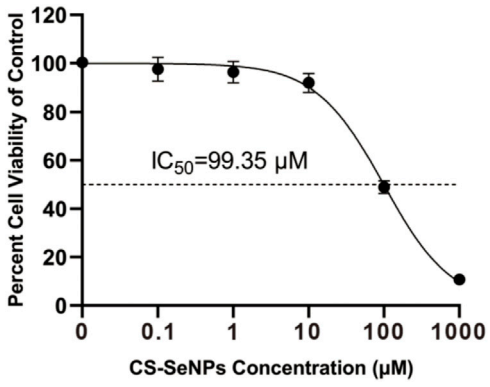

FIGURE 9
Cell viability of African green monkey kidney cell line MARC-145 on treatment using different concentrations (0–1000 μM) of chitosan–Nano-Se for 48 h by Cell Counting Kit-8 (Shao et al., 2022).

6 Challenges and prospects

SARS-CoV-2 infectious diseases present one of the major public health challenges of the 21st century. As compared to previous SARS-CoV-1 infection, SARS-CoV-2 infection has shown 40 times more transmission efficiency and a 3.3% higher fatality rate (Tiwari et al., 2022). SARS-CoV-2 variants have been causing continuous threat to public health and challenges to governments, health agencies, and researchers to end this pandemic. Therefore, researchers are urgently exploring different materials for the use in efficient COVID-19 pandemic management (Kaushik et al., 2020; Mujawar et al., 2020; Yalcin and Kaushik, 2021). In the past two decades, many efforts have been devoted by researchers in order to develop nanoselenium for various applications, including biological science and material science. However, there are still many knowledge gaps for the use of nanoselenium in the field of biomedical science. In the biomedical field, there are always challenges associated with exploring novel molecules (Figure 10).

Se is widely distributed in the human body in the form of 25 selenoproteins, which are important in many physiological processes. These selenoproteins are mainly involved in the

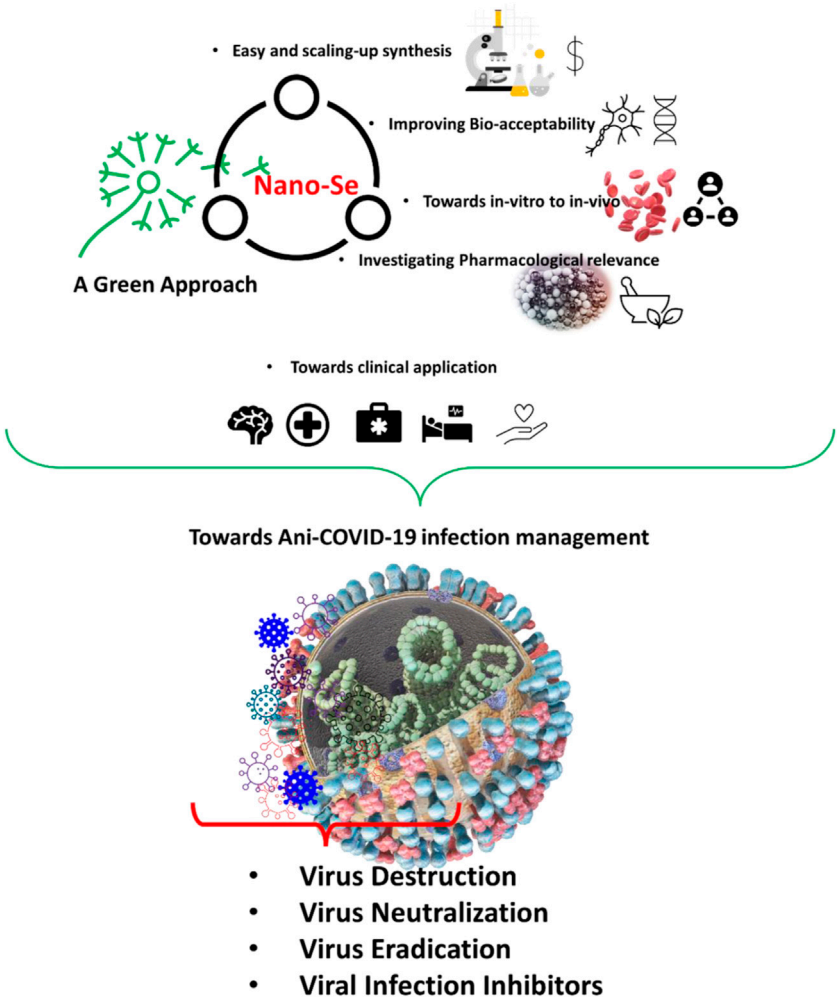

FIGURE 10
Prospects of Nano-Se toward using a green approach for efficient biomedical application, especially with a focus on tackling SARS-CoV-2 to manage COVID-19 infection management.

regulation of oxidative stress through antioxidative properties. The hypothesis that synthetic selenium species may be superior anti-oxidants than the natural antioxidants has led to research on selenium. Regarding synthetic selenium species, the first issue which comes to mind is the toxicity of selenium species. Different forms of selenium have vastly different toxic potentials. Nano-Se and related systems show excellent biological properties together with their lower toxicity. In order to really use nanoselenium to tackle muted SARS-CoV-2 for efficient COVID-19 management, it is necessary to have a clear and systematic investigation on easy scale-up synthesis using green approach, antiviral activity, mechanism study, and toxicology study. Green synthesis offers various advantages over chemical synthesis, such as ease in adopting large-scale production, easy handling, less-expensive materials, and eco-friendliness (Kalishwaralal et al., 2016; Dhawan et al., 2021). Furthermore, detailed *in vivo* studies with rigorous clinical trials and pharmacological studies are required to explore Nano-Se for biomedical applications (Figure 10).

7 Conclusion and viewpoint

This comprehensive review article explored the need for effective and efficient nanoassisted technologies to tackle mutated SARS-CoV-2 to manage COVID-19 infection. Since the emergence of the COVID-19 outbreak, policymakers, scientific communities, and health professionals have demanded a collaborative approach to develop effective approaches for ending this ongoing pandemic. Nanomaterials emerge as an important anti-SARS-CoV-2 agent, which can tackle SARS-CoV-2 *via* trapping, neutralizing, and eradication, and are required for efficient COVID-19 management even in a personalized manner. To achieve these tasks, we are propose and explore Nano-Se as an efficient

nanostructure capable of managing COVID-19 infection due to its excellent antiviral activity capable of neutralizing and eradicating SARS-CoV-2 variants. This article also raised the demand for additional efforts to promote acceptable and affordable Nano-Se as a part of COVID-19 infection management planning. Nano-Se can be a part of protective and treatment strategies in a personalized manner. However, further optimization is required (in laboratories, clinics, and community) considering FDA approval; therefore, a significant future research must be planned in this direction.

Author contributions

AS visualized and articulated the article. PS, RK, and AK contributed to editing, review, and finalizing the article.

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Conflict of interest

AS was employed by the company Molekule Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Isolation of SARS-CoV-2-blocking recombinant antibody fragments and characterisation of their binding to variant spike proteins

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COVID-19 is a severe acute respiratory disease caused by SARS-CoV-2. From its initial appearance in Wuhan, China in 2019, it developed rapidly into a global pandemic. In addition to vaccines, therapeutic antibodies play an important role in immediately treating susceptible individuals to lessen severity of the disease. In this study, phage display technology was utilised to isolate human scFv antibody fragments that bind the receptor-binding domain (RBD) of SARS-CoV-2 Wuhan-Hu-1 spike protein. Of eight RBD-binding scFvs isolated, two inhibited interaction of RBD with ACE2 protein on VeroE6 cells. Both scFvs also exhibited binding to SARS-CoV-2 Delta variant spike protein but not to Omicron variant spike protein in a Raman spectroscopy immunotest. The study demonstrates the potential of recombinant antibody approaches to rapidly isolate antibody moieties with virus neutralisation potential.

KEYWORDS

SARS-CoV-2, scFv, recombinant antibody, phage display, neutralisation, variant of concern

Introduction

COVID-19 disease is caused by the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It first emerged in China in late 2019 and was defined as a pandemic by the World Health Organisation (WHO) in March 2020. It is associated with high rates of morbidity and mortality, and over 608 million infections and 6.5 million deaths have been attributed to it worldwide in September 2022 (World Health Organization, 2022).

SARS-CoV-2 gains entry to host cells by attaching to the host angiotensin-converting enzyme 2 (ACE2) receptor *via* the virus homotrimeric spike (S) glycoprotein, leading to entry and replication in ACE2-expressing cells including lung, heart, intestine and kidney

cells. The spike protein is composed of domains S1, which contains the host receptor-binding domain (RBD) and an N-terminal domain (NTD), and S2, which mediates virus-cell membrane fusion (Letko et al., 2020). RBD is highly dynamic and alternates between a receptor-accessible "up"/"open" and a receptor-inaccessible "down"/"closed" state (Wrapp et al., 2020). Numerous studies of previously infected patients have identified antibodies against spike protein, and RBD in particular, which potently neutralise virus interaction with cells *in vitro* and prevent disease in animal models (Liu et al., 2020; Ke et al., 2022).

While the rapid development and roll-out of vaccines, at least in the developed world, greatly reduced the mortality associated with SARS-CoV-2, the development of therapeutics is also an important facet to lessen disease severity. This is particularly true in elderly or immunocompromised patients, or individuals with severe pre-existing conditions, in whom breakthrough infections can be life threatening (Beaudoin-Bussières et al., 2020; Jeyanathan et al., 2020; Havlin et al., 2021). Passive immunisation with antibodies against the virus spike protein has potential to neutralise SARS-CoV-2 (Yang et al., 2020) with immediate effect, rather than waiting for a number of weeks for active immunity to accrue from vaccination. A number of groups have reported the isolation of monoclonal antibodies which neutralise SARS-CoV-2 (reviewed in Corti et al., 2021; Hurt and Wheatley, 2021). Antibodies that have progressed to clinical trial stages to date are predominantly RBD-binding antibodies due to their high potency at blocking interaction of RBD with ACE2 (Li et al., 2022). Current details of monoclonal antibodies and antibody cocktails in Phase two or Phase three clinical trials for COVID-19 treatment or prophylaxis are available at https://www.antibodysociety.org/covid-19-biologics-tracker/.

Following FDA EUA approval of the use of convalescent plasma as a treatment for COVID-19 patients (Food and Drug Administration, 2020), studies confirmed the clinical benefit of administering COVID-19 convalescent plasma to hospitalised patients early in the course of disease (Casadevall et al., 2020). This led to U.S. Food and Drug Administration emergency use authorisations for a number of monoclonal antibodies and antibody combinations to reduce mortality in COVID-19 patients (Kumar et al., 2021; www.fda.gov). While a number of antibodies have demonstrated high efficacy in trials, their high administration doses (0.5–1.2 g per antibody per dose; Kumar et al., 2021) place considerable pressure on mammalian cell production pipelines, limiting their widespread use in disease treatment (Yang et al., 2021).

Therapeutic antibodies are traditionally produced *via* immunisation approaches, or reverse transcriptase-polymerase chain reaction (RT-PCR) amplification from B cells isolated from infected individuals, followed by *in vitro* screening (Hwang et al., 2022). Considerations of ethical and regulatory requirements, immunogenicity, cost, scale-up and timeliness have led, however, to increasing use of *in vitro* approaches to isolate (and engineer) instead recombinant antibody fragments with similar or improved properties compared to monoclonal antibodies. Isolation approaches, such as phage display technology, involve identification of binders of a ligand of interest from large, synthetic libraries of antibody fragments, typically of human origin, in a process which mimics antigen selection and affinity maturation (Marks et al., 1991). The approaches typically utilise antibody fragments that retain the variable V regions (encompassing the binding pocket) of full-length immunoglobulin molecules, but contain few or no constant (C) domains that mediate effector functions *in vivo*–but are unnecessary for virus neutralisation. The single-chain fragment variable (scFv) fragments utilised in this study contain the variable heavy (V_H) and variable light (V_L) domains of an antibody, joined by a peptide linker (Wronska et al., 2016). These small-sized fragments are easily produced in expression hosts such as *Escherichia coli* that are considerably cheaper, more robust and produce higher yields than the mammalian cells used to express whole antibodies (Wronska et al., 2016). Additionally, protein engineering to rapidly tune antibody properties such as affinity and specificity is facilitated in *E. coli* (Kiguchi et al., 2020).

Since its appearance, SARS-CoV-2 continues to undergo significant antigenic drift, resulting in the emergence of several variants of concern with higher virulence and/or reduced neutralisation by therapeutic antibodies (Jeong et al., 2022; Sievers et al., 2022). Safe and effective therapies are therefore needed to combat the transmissibility and disease severity of new SARS-CoV-2 variants. The ability to rapidly isolate antibody fragments using *in vitro* screening technologies, and to effectively and cheaply scale-up production in recombinant expression platforms, enables a quick pivot to antibodies which neutralise emerging virus variants. This can form part of a core toolset to respond to emerging variants in this and future epidemics, by providing faster responses, easier manufacturability and lower cost therapeutics.

A variety of recombinant antibody fragments have been isolated against SARS-CoV-2 to date, using cloning approaches from antibodies from COVID-19 patients (Ebihara et al., 2021), antibody gene libraries derived from convalescent COVID-19 patients (Bertoglio et al., 2021; Mendoza-Salazar et al., 2022; Minenkova et al., 2022), or by immunising animals such as alpacas (Güttler et al., 2021). Phage display has also been utilised to isolate nanobodies (single-domain antibodies derived from camelids; Muyldermans et al., 2013) with SARS-CoV-2 neutralising ability (Zhao et al., 2022), as well as scFv-Fc fusion proteins against nucleocapsid protein for use in diagnostics (Kim et al., 2021). Numerous studies have also reported the use of phage display to isolate scFvs against RBD but without investigating their virus neutralisation ability (Salem et al., 2022; Parray et al., 2020).

Therefore, the goal of our work was to demonstrate rapid isolation of SARS-CoV-2-binding antibody fragments from a naïve human scFv antibody library and characterisation of their virus neutralisation potential. Phage display was utilised to identify fragments that bind spike protein RBD, followed by

screening for inhibition of the RBD-ACE2 interaction and investigation of binding of emerging virus variant spike proteins to determine the potential for cross-reactive neutralising ability. The work demonstrates the potential of recombinant antibody approaches to rapidly develop virus-neutralising antibodies for use in passive immunotherapy.

Materials and methods

Materials

All chemicals were from Sigma-Aldrich unless otherwise specified. *E. coli* strain HB2151 was used for soluble expression of scFvs. The YamoI human scFv library was from Montarop Yamabhai (Pansri et al., 2009). SARS-CoV-2 proteins and inactivated viruses from BEI Resources (Manasses, Virginia) are listed in the Acknowledgements. The Omicron (B.1.1.529) variant spike trimer was purchased from Sino Biological (cat. #40589-V08H26).

ELISA analysis

Procedures for phage library panning were as described previously (Antoine et al., 2022). After three rounds of panning, eluted phages were rescued and titred in *E. coli* TG1 cells for RBD-binding assessment by ELISA. Wells of a 96-well microtitre plate (Maxisorb, Nunc) were coated overnight at 4°C with 2 µg/ml of RBD (BEI Resources) in PBS. After 3 washes with PBS, wells were blocked using PBS/4% bovine serum albumin (BSA) for 2 h at room temperature, followed by five washes with PBS. Wells were incubated with 100 µl of scFv-phage or soluble scFvs for 1 h at room temperature, and washed three times with PBS/0.1% Tween-20 and three times with PBS. After 1 h incubation at room temperature with anti-M13 horseradish peroxidase (HRP)-conjugated IgG (0.4 µg/ml in PBS/1% BSA) for scFv-phage analysis, or anti-c-myc HRP-conjugated IgG (Abcam, United Kingdom; diluted 1:250 in PBS/1% BSA) for soluble scFv analysis, the wash step was repeated and 100 µl of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added. Reactions were stopped using 100 µl 1 M H_2SO_4 and absorbances were read at OD_{450}. Half maximal effective concentrations (EC_{50}) were calculated with GraphPad Prism Version 9.3, utilising fitting to a four-parameter logistic curve.

ScFv expression

Plasmid DNA was purified from individual *E. coli* TG1 clones expressing RBD-binding scFv-phage and used to transform *E. coli* HB2151 cells. Overnight *E. coli* cultures were used to inoculate 50 ml of ZY-5052 autoinducing medium containing 100 µg/ml ampicillin to a starting OD_{600} of 0.05. Supernatants from 50 ml cultures were collected by centrifugation to pellet cells after 48 h induction at 37°C and 225 rpm, followed by purification by immobilised metal affinity chromatography (IMAC) (Antoine et al., 2022).

ScFv purification

For IMAC purification of scFvs, a 1 ml Hitrap column (GE Healthcare, United Kingdom) was loaded with Ni^{2+} before equilibration with wash buffer (20 mM sodium phosphate, 0.5 M NaCl, pH 7.4) containing 20 mM imidazole. Protein samples were filtered through a 0.4 µm filter, followed by addition of 20 mM imidazole before loading onto the column. The column was washed with 20, 30 and 10 ml wash buffer containing 20, 50 and 70 mM imidazole, respectively, and eluted using wash buffer containing 400 mM imidazole. Eluted fractions were dialysed against PBS, and purified proteins were analysed under denaturing conditions in 12% SDS-PAGE, with staining with InstantBlue Coomassie stain (Expedeon, United Kingdom). Protein concentration was determined using a BCA Assay kit (Thermo Fisher Scientific, United Kingdom).

DNA sequencing and analysis

ScFv diversity was analysed by *Bst*NI restriction analysis of plasmid DNA from RBD-binding clones and electrophoresis on 1.3% agarose gels. ScFv genes were sequenced at Eurofins Genomics (Ebersberg, Germany).

Inhibition of RBD binding to ACE2-expressing cells

Investigation of inhibition of RBD-ACE2 binding was performed using flow cytometry. For each scFv, 3 µg/ml of SARS-CoV-2 Spike RBD Alexa Fluor 647 (Bio-Techne, Abingdon, United Kingdom) was added to varying concentrations of scFv in a 100 µl final volume and incubated for 1 h at room temperature. The RBD-scFv mixture was incubated with 1×10^5 ACE2-expressing Vero E6 cells (Thermo Fisher Scientific) for 1 h at room temperature. Cells incubated with RBD Alexa Fluor 647 alone were used as the negative control. Positive control cells were pre-incubated with 100 µL of 3 µg/ml unlabelled RBD (BEI Resources) for 1 h at room temperature to block RBD binding sites of ACE2 prior to addition of the RBD Alexa Fluor 647. After three washes with 2% fluorescence-activated cell sorting (FACS) buffer (PBS supplemented with fetal calf serum), the fluorescence of positive cells was measured using a BD Accuri C6™ flow cytometer.

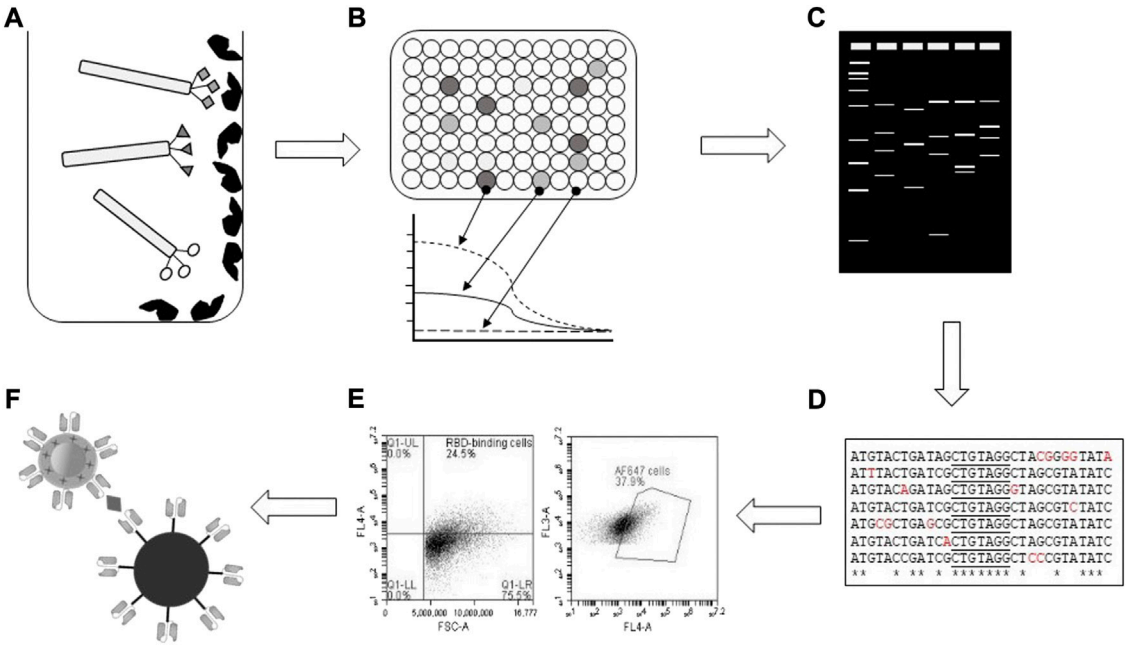

FIGURE 1
Schematic of experimental workflow. **(A)** RBD-binding bacteriophage-scFvs were selected based on binding to immobilised RBD *in vitro*, followed by **(B)** ELISA confirmation of binding and EC_{50} determinations. **(C)** Restriction profiling of isolated RBD-binding clones identified unique patterns for **(D)** DNA sequencing and bioinformatic analysis. **(E)** Flow cytometry screening identified scFvs which blocked RBD interaction with ACE2-expressing Vero E6 cells, with **(F)** screening for binding to variant spike proteins carried out by immunocomplex formation and SERS Raman spectroscopy.

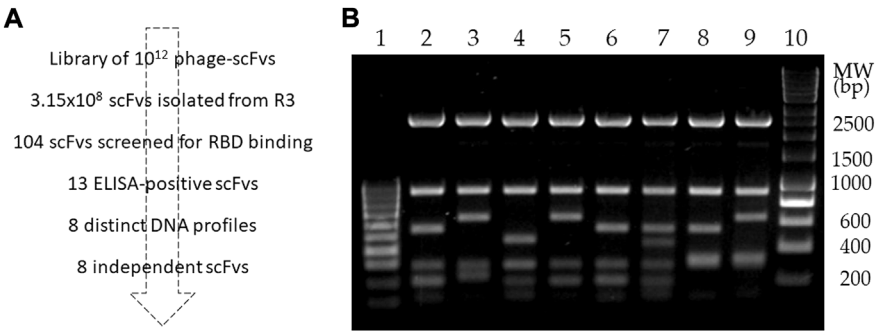

FIGURE 2
Isolation and DNA fingerprinting of RBD-binding scFvs. **(A)** Workflow showing isolation of RBD-binding scFvs. **(B)** Restriction profile analysis of eight RBD-binding scFvs isolated from library screening. Lanes: 1, 10: molecular weight markers; 2–9: *Bst*NI-digested DNA from scFvs 2, 3, 10, 37, D4, E10, F11 and G12, respectively.

Binding of variant spike proteins

ScFvs were evaluated for binding of SARS-CoV-2 variant spike proteins using an immunoassay previously described (Antoine et al., 2022). SERS nanotags, which consist of an scFv conjugated to 60 nm gold nanoparticles, were generated by a layer-by-layer method as described elsewhere (Antoine et al., 2022), while scFvs were conjugated to magnetic particles (Pierce NHS-Activated Magnetic Beads, cat. #88826, Invitrogen) following the manufacturer's protocol. SERS immunoassays were carried out by combining scFv-conjugated magnetic beads, scFv-conjugated SERS nanotags and 50 pg of target antigen in PBS containing 1% BSA in a final volume of 500 µl, followed by incubation at room temperature with shaking at 200 rpm for 20 min and pelleting of

immunocomplexes using an external magnet (Antoine et al., 2022). For single-scFv assays, the same scFv was conjugated to both magnetic beads and SERS nanotags; for two-scFv assays, scFv 10 was conjugated to magnetic beads and scFv D4 to SERS nanotags. Raman spectra of pellets were measured with a Mira DS handheld Raman spectrometer (Metrohm). Spectra were collected using a laser power of 5 (50 mW), integration time 1 s, and raster off, and the peak height was measured at 591 cm^{-1}. Assays were performed in triplicate, and data is represented as the average of those triplicates with standard deviations.

Results

ScFv isolation and sequence analysis

After plating of phage clones isolated from Round 3 of the panning (Antoine et al., 2022), 104 clones were screened for binding to RBD (Figure 1). Of these, 13 positive clones were identified, which yielded eight distinct profiles upon restriction analysis using *Bst*NI (Figure 2). After confirmation by PCR of the presence of a gene of the expected size of scFvs in each clone, DNA sequencing identified the sequence of the eight different scFvs.

Recombinant protein expression

The eight RBD-binding scFvs were produced in soluble, non-phage-displayed form in 50 ml culture scale using an optimised autoinduction method utilising accumulation of scFvs in the extracellular medium, with equivalent yields after purification by IMAC ranging from 0.5 to 40 mg per liter of bacterial culture (Table 1). The binding of each purified scFv to RBD was confirmed by ELISA (data not shown) prior to RBD-ACE2 inhibition analysis.

TABLE 1 ScFv production yields in *E. coli*.

scFv	Yield (mg/L)
2	30
3	40
10	1.5
37	14
E10	40
F11	5
D4	10
G12	0.5

Production yields of scFvs per litre of *E. coli* HB2151 culture after purification by immobilised metal affinity chromatography (IMAC)

RBD-ACE2 binding inhibition

The ability of the purified scFvs to inhibit binding of RBD to ACE2-positive Vero E6 cells was assessed in a cell-based assay in order to identify potential neutralising molecules. A fluorochrome-conjugated RBD was used in the place of SARS-CoV-2 virus and allowed precise gating of positive cells during the flow cytometry analysis (Supplementary Figure S1). Using a scFv:RBD molar ratio of 100:1, two scFvs were found to inhibit RBD binding to the ACE2-expressing cells by more than 50%: scFv 10 reduced RBD binding to cells by 55%, while scFv D4 inhibited 94% of RBD-cell binding compared to the no-scFv control (Figure 3A).

To further characterise the neutralisation potential of scFvs D4 and 10, their ability to inhibit RBD binding to ACE2-expressing Vero E6 cells was measured over a concentration range of 2–530 μg/ml scFv in a cell-based flow cytometry assay (Figure 3B). While scFv D4 showed a half-maximal inhibitory concentration (IC_{50}) of RBD binding of 79 μg/ml, signal saturation was not achieved at 530 μg/ml for scFv 10. However, 56% inhibition was measured at 530 μg/ml scFv 10. Meanwhile, half-maximal effective concentrations (EC_{50}) of 11 μg/ml and 280 μg/ml were determined by ELISA for scFvs D4 and 10, respectively, for SARS-CoV-2 RBD (data not shown). The EC_{50} of scFv D4 for spike protein was also measured and was similar to the EC_{50} for RBD, at 8 μg/ml.

ScFv binding of variant spike proteins

To determine the potential of the neutralising scFvs raised against Wuhan-Hu-1 SARS-CoV-2 RBD to also block host cell interactions of emerging virus variants, the scFvs were also screened for their binding to Delta and Omicron spike proteins. Both scFvs exhibited binding to spike protein from the Delta variant, albeit with SERS signals reduced by 26% and 14% for scFv D4 and scFv 10, respectively. No binding of the Omicron variant protein was detectable for either scFv, however (Figure 4).

Discussion

The ongoing COVID-19 pandemic has crystallised the need for rapid isolation of virus-binding moieties, both for use in diagnosis, in order to identify and isolate potentially infectious individuals and thereby reduce spread of the disease, and for treatment, in order to mitigate the severity of the disease in infected and particularly high-risk individuals (Zhu et al., 2020).

Antibodies occupy a central role in the detection and identification of pathogens due to their natural binding specificity and affinity for their targets. In addition, the ability of antibodies to block or outcompete specific binding interactions

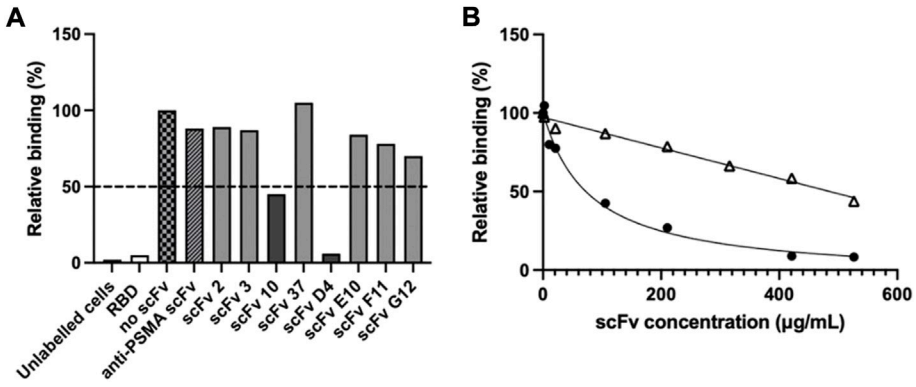

FIGURE 3
Inhibition of RBD binding to ACE-2 cells by scFvs. Flow cytometry was used to determine inhibition of RBD-ACE-2 binding. **(A)** Alexa Fluor 647-labelled SARS-CoV-2 RBD was pre-incubated with scFv (100:1 scFv:RBD ratio), prior to incubation with ACE-2-expressing Vero E6 cells. Unlabelled cells, cells pre-incubated with unlabelled RBD ("RBD"), cells pre-incubated with Alexa Fluor 647-labelled RBD but no scFv ("no scFv"), cells pre-incubated with Alexa Fluor 647-labelled RBD and an irrelevant "anti-PSMA scFv" were used as controls. Values show single measurements. The dotted line indicates 50% inhibition of RBD-cell binding. **(B)** IC_{50} determination of RBD binding to ACE-2 cells with inhibitory scFvs 10 (△) and D4 (●). 80 nM Alexa Fluor 647-labelled SARS-CoV-2 RBD was pre-incubated with scFv 10 or scFv D4 prior to addition to Vero E6 cells. Cells incubated with RBD-Alexa Fluor 647 without scFv were used as a control. Values show single measurements. The IC_{50} was calculated with GraphPad Prism version 9.3, using fitting to a four-parametric nonlinear regression curve.

has led to their use in neutralising viruses and toxins (Cheedarla and Hanna, 2019). As the classical hybridoma technology developed with murine B cells (Köhler and Milstein, 1975) has proven difficult to adapt to the generation of human monoclonal antibodies, however, recombinant approaches have been widely developed to create human antibodies, or derived antibody fragments (Wronska et al., 2016), which can be used *in vivo* in applications such as drug delivery and neutralisation, and *in vitro* methodologies like immunodiagnosis (Raeisi et al., 2022; Wang et al., 2022).

While the Fc region of antibodies–which is absent from the scFv fragments utilised in this study–is essential for many *in vivo* functions such as antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis and complement activation, recombinant fragments containing only V regions can effectively neutralise viruses and toxins by blocking their interaction with cells. In the case of SARS-CoV-2, Fc effector functions have been demonstrated to not be required for optimal protection against lethal infections in transgenic mice (Noy-Porat et al., 2021). This ability to neutralise SARS-CoV-2 in the absence of antibody C domains is important as it allows the use of *E. coli* expression systems for production and scale-up of small-sized recombinant fragments that do not require glycosylation, resulting in faster production, higher yields and reduced costs than for traditional monoclonal antibody production–all critical considerations when rapidly responding to an infection. The biophysical properties of antigen specificity and affinity are typically retained in recombinant antibody fragments compared to monoclonal antibodies from mammalian cells–with the added advantage of the ease of affinity maturation of fragments routinely allowing selection of molecules with affinities 10–50 times higher than their parent antibodies (Marks et al., 1992). While scFvs retain the intramolecular disulfide bonds of whole antibody V_H and V_L regions, they are nevertheless more prone to thermally-induced aggregation (Jager and Plückthun, 1999), which can be reduced using directed evolution or rational design approaches (Kang and Seong, 2020). Similarly, while their smaller size leads to faster clearance than whole antibody molecules *in vivo*, protein engineering can be used to extend their *in vivo* half-life and increase their pharmaceutical efficacy (Esquerda-Canals et al., 2019; Seifert and Kontermann, 2022).

E. coli can also have difficulty in expressing some mammalian proteins, leading to their accumulation in inclusion bodies or protein degradation, while it also struggles with multiple disulfide bridges and cannot glycosylate protiens (Rosano and Ceccarelli, 2014). ScFv yields in this study were as high as 40 mg/L in simple batch cultures, however, though yields can typically be further increased by approaches such as process optimisation or molecular chaperone overproduction (Hu et al., 2007), or particularly by adoption of a fed-batch culture system to increase cell density (Gąciarz et al., 2017).

Only two of the eight scFvs, which were isolated from the library based on their RBD binding, prevented RBD from interacting with the ACE2 receptor in a cell-based assay. The RBD domain used in biopanning is a 25-kDa protein containing 223 amino acids but only 17 amino acid residues make contact with (*i.e.* occur within a distance cut-off of 4 Å of) the

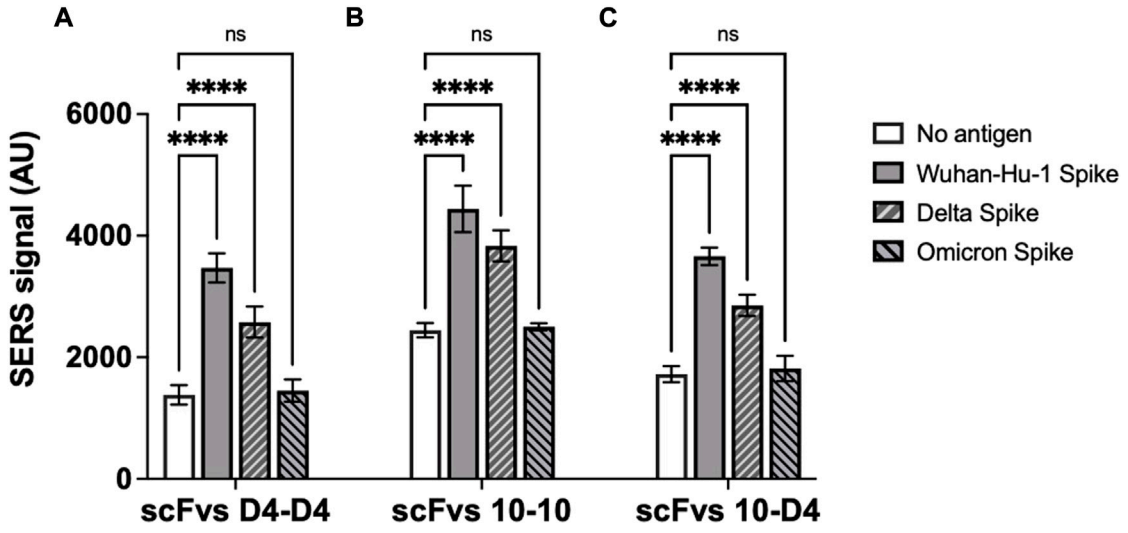

FIGURE 4
ScFv D4 and scFv 10 binding to spike proteins from SARS-CoV-2 Wuhan-Hu-1 virus and Delta and Omicron variants. Panels represent assays carried out with **(A)** scFv D4 on both magnetic nanoparticles and SERS nanotags, **(B)** scFv 10 on both particles, and **(C)** scFv 10 on magnetic nanoparticles with scFv D4 on SERS nanotags. Values represent the average of three replicate wells, and error bars indicate the standard deviation. For statistical analysis, two-way ANOVA followed by Dunnett's multiple comparisons test was performed: ****: $p < 0.0001$; ns: not significant.

ACE2 receptor (Lan et al., 2020). Therefore, it is unsurprising that most of the isolated RBD-binding scFvs did not bind to the domain at a site that impedes its interaction with ACE2.

ScFv D4 was particularly effective at inhibiting the interaction of RBD with ACE2-expressing cells, indicating its binding target is likely to overlap more completely with the ACE2 interaction site than scFv 10. RBD epitopes are categorised into four classes, with Classes 1 and 2 overlapping the ACE2 binding site and thereby constituting the major target for neutralising antibodies (Onodera et al., 2021). Many neutralising molecules have been demonstrated to bind at, or close to, the receptor binding motif of the RBD and inhibit its attachment to the ACE2 protein (Xiang et al., 2020; Hanke et al., 2022), which is necessary to infect host cells, or lock the RBD in its "down" conformation to prevent productive RBD-ACE2 fusion (Schoof et al., 2020).

The differing neutralisation properties of the two RBD-ACE2-blocking scFvs is suggestive of different binding epitopes in RBD. Investigation of the predicted amino acid sequences of the two scFvs revealed only 27% identity and 64% similarity between their V_H CDR sequences, compared to 66% and 89%, respectively, across their entire gene sequences (not shown). As the use of multiple neutralising molecules binding different epitopes can be used in combination therapies to mitigate mutagenic escape in newly emerging virus variants (Makdasi et al., 2021), further investigation will be necessary to determine the precise epitopes bound by the two scFvs. This cocktail approach is seen in the emergency use authorisation (EUA) by the U.S. FDA of commercial monoclonal antibody combinations for treatment of SARS-CoV-2 infections (Baum et al., 2020; Bennett et al., 2021). Furthermore, simultaneous targeting of more than one epitope in a diagnostic test could be used to improve detection of virus, including new VOCs, and, importantly, to rapidly distinguish between VOCs in a point-of-care test.

While both of the present scFvs retained binding to spike protein from the Delta variant virus, neither scFv exhibited detectable binding of Omicron spike protein. This contrasts with scFv 3 which was also isolated from the biopanning and bound Alpha, Beta, Delta and Omicron spike proteins with similar affinity (Mohammadi et al., 2022), but failed to inhibit the RBD-ACE2 interaction in this study. The inability of the Wuhan-Hu-1- and Delta-binding scFvs 10 and D4 to detect Omicron spike protein mirrors clinical experience, with the ongoing emergence of virus variants which are resistant to detection and/or neutralisation by antibodies protective against earlier strains (Focosi et al., 2021). The large number of amino acid substitutions in Omicron in particular (37 in spike glycoprotein, compared to approximately 10 in Alpha and Beta variants of concern (VOCs), and 15 mutations in the RBD alone (Ou et al., 2022)) has exacerbated its evasion of immunoprotection from previous infections. Many Class 1 RBD-binding antibodies, with epitopes centred around loop 477–489, are frequently subject to Omicron escape due to mutations in this protein region, though neutralisation towards Omicron may be retained by antibodies which target the highly conserved SD1 region of the spike protein (Hong et al., 2022).

This study outlines an approach to rapidly isolate scFvs against current SARS-CoV-2 virus variants, followed by screening of the scFv panel for binding and neutralisation of newly emerging VOCs, with *de novo* biopanning against new variant proteins in 3–4 weeks if necessary. This provides a promising strategy to isolate detection and treatment moieties which can be more rapidly tailored to detect or treat new variants in immunocompromised, young or elderly individuals than monoclonal antibody-based reagents.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

PJ, KW, and GW contributed to conception and design of the study. DA, MM, CM, and EW carried out the experimental work. DA, MM, PJ, KW, and GW performed the data analysis. DA and GW wrote the first draft of the manuscript. PJ, KW, and GW acquired funding for the work. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnano.2022.1028186/full#supplementary-material

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Prospects of TiO_2-based photocatalytic degradation of microplastic leachates related disposable facemask, a major COVID-19 waste

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COVID-19 is one of the serious catastrophes that have a substantial influence on human health and the environment. Diverse preventive actions were implemented globally to limit its spread and transmission. Personnel protective equipment (PPE) was an important part of these control approaches. But unfortunately, these types of PPE mainly comprise plastics, which sparked challenges in the management of plastic waste. Disposable face masks (DFM) are one of the efficient strategies used across the world to ward off disease transmission. DFMs can contribute to micro and nano plastic pollution as the plastic present in the mask may degrade when exposed to certain environmental conditions. Microplastics (MPs) can enter the food chain and devastate human health. Recognizing the possible environmental risks associated with the inappropriate disposal of masks, it is crucial to avert it from becoming the next plastic crisis. To address this environmental threat, titanium dioxide (TiO_2)-based photocatalytic degradation (PCD) of MPs is one of the promising approaches. TiO_2-based photocatalysts exhibit excellent plastic degradation potential due to their outstanding photocatalytic ability, cost efficiency, chemical, and thermal stability. In this review, we have discussed the reports on COVID-19 waste generation, the limitation of current waste management techniques, and the environmental impact of MPs leachates from DFMs. Mainly, the prominence of TiO_2 in the PCD and the applications of TiO_2-based photocatalysts in MPs degradation are the prime highlights of this review. Additionally, various synthesis methods to enhance the photocatalytic performance of TiO_2 and the mechanism of PCD are also discussed. Furthermore, current challenges and the future research perspective on the improvement of this approach have been proposed.

KEYWORDS

COVID-19, face mask, microplasitcs, waste management, TiO_2, photocatalytic remediation

1 Introduction

Personnel protective equipment (PPE) plays a crucial role in safeguarding individuals during COVID-19 (Benson et al., 2021). PPE comprises disposable face masks (DFMs), gloves, goggles, gowns, face shields, respirators, and aprons made largely of single-use plastics, according to WHO (2020). Wearing DFM is one of the effective strategies against COVID-19 (Vieten, 2020; Du et al., 2022a). These face masks mainly comprise three layers: an inner layer of soft fibers, a middle layer of a melt-blown filter, and an exterior covering of nonwoven fibers that ensures water resistance (Fadare and Okoffo, 2020). Polypropylene (PP), polystyrene (PS), polycarbonate (PC), polyurethane, polyethylene (PE), and polyacrylonitrile (PAN) are the most common polymers that are used in face masks (Akber Abbasi et al., 2020).

Since the commencement of the pandemic, the quantity of litter produced by the COVID-19 outbreak such as DFMs, and other PPE has increased dramatically. Especially, this outbreak has resulted in an astounding increase in the usage of DFMs to prevent disease transmission (Sullivan et al., 2021). The handling of wastes generated by masks is a troublesome reflection of the COVID-19 outbreak, which has devastated worldwide healthcare systems and affected national economies (Herron et al., 2020; Patrício Silva et al., 2020). In early 2020, WHO anticipated that 89 million DFMs will be needed per month for health workers (de Sousa, 2021). China's daily manufacturing of DFM has increased to 14.8 million during (February) 2020. According to US officials, 3.5 billion masks will be needed by their nation to combat this pandemic (de Sousa, 2021). It was predicted that more than 7 billion masks would be used every day throughout the world (Hantoko et al., 2021). Moreover, it was anticipated that by 2020, 1.56 billion (5,159–6,878 tons) of plastics (only from COVID-19 DFM) might leach into oceans (Shams et al., 2021). In the United Kingdom (66.7 million residents), it was estimated that if every resident wore one mask every day, a minimum of 60,000 tons of plastic trash would be generated (Benson et al., 2021). In Brazil, it is estimated over 85 Million used masks might be discarded each day (Urban and Nakada, 2021). It is projected that 0.15 million–0.39 million tons of mishandled COVID-19 plastic trash might wind up in the global oceans within a year (Chowdhury et al., 2021; Patrício Silva et al., 2021). Even if only 1% of disposable face masks were discarded improperly by the global population, it would result in the release of 10 million (30,000–40,000 kg) masks into the environment (WWF International, 2020). There have been numerous reports of used DFMs cluttering city streets, flowing through sewage lines, and floating in seas right from the onset of the COVID-19 outbreak (Ardusso et al., 2021; Okuku et al., 2021; Torres and De-la-Torre, 2021). The release of microplastics from face masks was reported in the recent literature (Saliu et al., 2021; Sullivan et al., 2021; Wang et al., 2021b). Physiochemical processes such as UV radiation, wind, currents, and other biochemical processes in the environment trigger the disintegration or degradation of these used masks into nanofibers and/or microplastics (MPs) (<5 mm) (Du et al., 2022a). Owing to the remarkable resistance of the plastics, they are inconceivable for complete mineralization and biological breakdown. Consequently, most plastics will remain in the environment for a longer period (Khoo et al., 2021) and endanger the wellness of the ecosystem (Kane et al., 2020; Benson et al., 2021). As the size of the plastics reduces, they are more likely to be ingested and accumulated by the organisms which subsequently creates a high chance of entry into the food chain. Recent studies have demonstrated that certain MPs and nanoplastics can be absorbed by the stomach and passes across the blood-brain barrier, causing neurotoxic injury (Prust et al., 2020). This enormous increase in DFM waste and other PPE may add to the avalanche of plastic pollution (Benson et al., 2021). Particulate matter (PM) is one of the influencing factors which have contributed to the increased spread of COVID-19. PM could generate a condition suitable for spreading the virus over larger distances than those envisaged for intimate contact (Comunian et al., 2020). Apart from the usage of DFMs to combat COVID-19, it is also used to prevent air pollution (Morgana et al., 2021; Chaudhary et al., 2022). This resulted in increased mask waste generation, which makes it crucial to explore all potential environmental consequences. Photocatalysis is one of the remarkable measures for the degradation of MPs leachates that are released from DFMs. This approach is reliable and affordable as it utilizes sunlight. It is a promising approach mainly due to its low cost and great efficiency when compared to other approaches (Mandade, 2021). The underpinnings of photocatalytic degradation (PCD) are photocatalysts with strong redox potential. Titanium dioxide (TiO_2) is considered the quintessential photocatalyst because of its unique qualities such as biocompatibility, high stability, availability, low operating temperature, and low cost (Turkten and Bekbolet, 2020). This approach has the potential for the degradation of various organic pollutants including microplastic leachates from DFMs.

2 COVID-19 and the untenable waste management

Plastic pollution has increased as a result of poor plastic waste management (PWM) during the COVID-19 outbreak throughout the world (Patrício Silva et al., 2021). Though PPE may be a lifesaver during COVID-19, the accumulation, mishandling, and dumping of these PPE wastes resulted in a sudden collapse of waste management chains. This leads to catastrophic plastic pollution in the ecosystem. The COVID-19 pandemic has enhanced the complexity of PWM and appears to be impeding the attempts to eliminate plastic pollution.

During COVID-19, there was an unconvincing increase in global medical waste from 200 tons/day to 29,000 tons/day in a short period (February 2020–September 2020). During COVID-

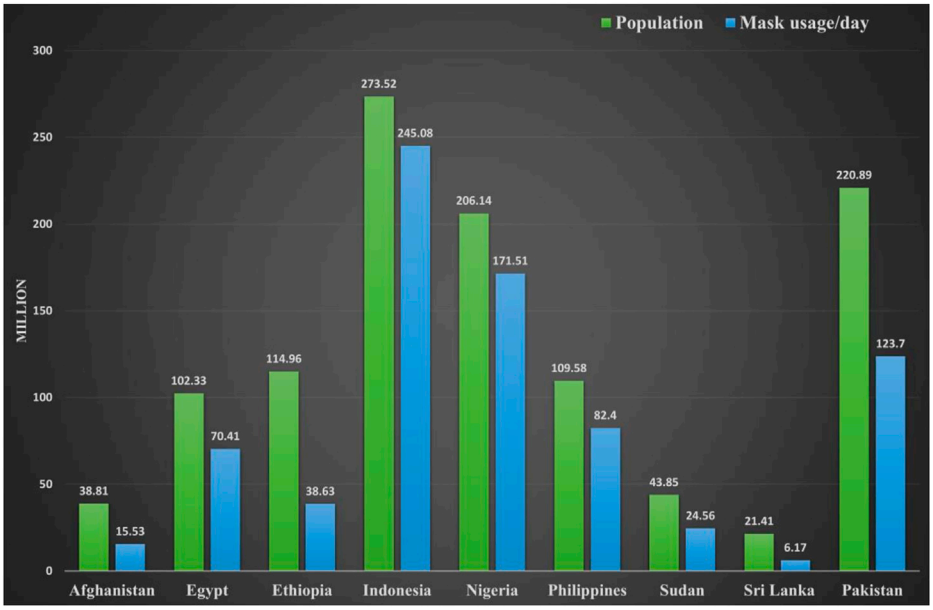

FIGURE 1
Population and mask usage rate in some developing countries.

19 tests, kits including plastic items such as tips, pipettes, falcon tubes, 96 well plates, Eppendorf tubes, and optical plastics plates were utilized. As a consequence of this increased usage, it was predicted that about 37 g of plastic debris will be left in the environment for each test (Celis et al., 2021). Medical waste surged from 45 to 247 tons (about 6 times higher when compared to pre-COVID) in China; a 30%–50% increase in the most afflicted districts of France; and a 30% surge in the Netherlands (de Sousa, 2021). In China, the Ministry of Ecology and Environment reported a 23% increase in the quantity of biomedical waste produced during the initial phase of the COVID-19 outbreak (Parashar and Hait, 2021).

The increase in DFM wastes is viewed as a new source of pollution that is intimately linked to the COVID-19 outbreak (Sullivan et al., 2021). Even in less populous nations like Ireland (almost 4.5 million people), health personnel utilized 9 million masks every week (Liang et al., 2021). The disposal of used DFMs appears to be substantially greater on rivers and beaches. Beaches in Kenya reported ten times more dumping of used masks than streets (Okuku et al., 2021). It was estimated that around 250 DFMs may enter aquatic systems each day in Jakarta, Indonesia (Cordova et al., 2021). Used masks have been littered in metropolitan areas, with concentrations of about 0.001 items per square meter in Peru and Canada, and, it was less than 0.3 items per square meter in Kenya (Ammendolia et al., 2021; De-la-Torre et al., 2021). The use of DFM surged in most of the developing nations. Population and predicted mask usage rate (per day) in some developing nations as predicted by Badillo-Goicoechea et al. (2021) is depicted in Figure 1. This exorbitant usage of various PPE (especially DFM) and other medical-associated plastics has elevated the complication in managing COVID-19 wastes.

2.1 Limitations of current techniques

All over the world, widely employed approaches in plastic waste management are landfilling, incineration, and mechanical recycling (Alabi et al., 2019). The majority of plastic waste can be disposed of through incineration and landfills. The main issue is that it demands a huge space and energy (Anderson et al., 2021). The incineration of these biohazardous wastes will lead to air pollution, raising the particulate matter in the air and boosting the risk of COVID-19 infection and other respiratory issues (Torres-Agullo et al., 2021). During COVID-19, landfills were overloaded with plastic waste beyond their capacity. This increased landfill dumping might lead to various adverse effects including the leaching of adverse chemicals (Selvaranjan et al., 2021; Shams et al., 2021). The astounding generation of plastic waste combined with the lack of recycling measures during the lockdown resulted in a significant decline in global plastic recycling. The vast majority of plastics can be recycled and reused. Manpower is essential in the collection and sorting of plastics. But, one of the primary challenges with plastic recycling during COVID-19 was the lack of manpower as people were hesitant to collect COVID-19-based wastes (Anderson et al.,

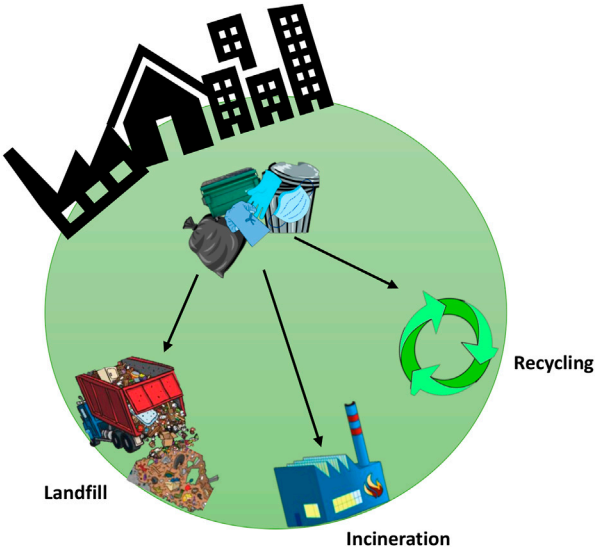

FIGURE 2
Major approaches in the disposal of plastic wastes.

2021). The most difficult components of recycling are collecting and sorting plastic trash. Moreover, mechanical recycling is fraught with difficulties, such as inorganic pollutants, additives, and polymer cross-contamination (Anderson et al., 2021). Incineration can emit toxic gases such as dioxins and furans into the atmosphere, which paves way for global warming (Torres and De-la-Torre, 2021). The widely employed approaches in plastic disposal are depicted in Figure 2. In terms of MPs removal, wastewater treatment plants (WTP) are not satisfactory. They may easily be eluded from the collection by WTP due to their smaller size. These MPs may eventually result in the aquatic ecosystem. Studies on WTP from various sites revealed that an excessive quantity of MPs in the treated water ranges between 5.00×10^5 and 1.39×10^{10} particles (Hamd et al., 2022). As per the scientific reports, there is still no distinct approach in any WTP for the elimination of MPs. Ideally, these disadvantages make these processes not suitable for sustainable plastic waste degradation.

3 Environmental impact of microplastics generated from disposable face masks

The nature of deposition sites and composite materials determine the fate of the DFMs (Du et al., 2022a). Environmental factors trigger the transformation of used DFMs into MPs, which are considered as a new form of environmental pollutant. In most cases, used DFMs are often thrown randomly or segregated as plastic waste. These wastes are either incinerated or disposed of in landfills (Du et al., 2022a). Because of the inclusion of plastics in DFMs and its subsequent waste mismanagement, there are numerous negative environmental consequences. In general, terrestrial ecosystems are the primary sources of aquatic plastic pollution, which is mostly due to anthropogenic sources. About 80% of worldwide ocean plastics originate from land, with the remaining 20% ascribed to marine sources (LI et al., 2016). The impact of mask-generated MPs on terrestrial and aquatic ecosystems is depicted in Figure 3.

3.1 Terrestrial ecosystem

3.1.1 Impact on animals

Face masks that have been dumped in the soil can endanger fauna causing entanglement and even mortality (Hiemstra et al., 2021; Patricio Silva et al., 2021; Du et al., 2022a; Yang et al., 2022). Several incidents of animals becoming tangled in discarded facemasks have been documented across the world including entanglement in claws, snouts, necks, legs, and other parts of the body (Hiemstra et al., 2021; Patrício Silva et al., 2021). DFMs are also reported to be used by some birds as nesting material. This may alter the thermal conditions and increase the likelihood of ingestion or entanglement (Hiemstra et al., 2021). Reports suggest that hedgehogs, American robins, swans, bats, mallards, and gulls are at significant risk of being the victims of masks and other PPE entanglement (Yang et al., 2022). Most of the DFMs are disposed of as solid waste, and animals that rely on landfills for food may be particularly vulnerable to plastic uptake. For example, in Spain, *Ciconia* (white storks) were discovered to feed on landfill trash accounting for 68.8% of their diet (Peris, 2003). Moreover, when animals mistook face masks for food, these plastics can block their intestines, impede food intake, and cause a slew of health issues, including death (Du et al., 2022a). For example, a significant amount of landfill debris was also found in the guts of overwintering gull species like *Larus marinus*, *L. glaucoides*, and *L. smithsonianus* (Seif et al., 2018; Patrício Silva et al., 2021).

3.1.2 Impact on plants

Once dumped on the soil, plastic debris may clog sewage systems and leads to various detrimental effects on the soil. It impacts agricultural water percolation, soil aeration, and reduces soil quality (Prata et al., 2020). Wet-dry cycles, plowing, and other bio-factors, rapidly move MPs into the soil and spoil the soil structure, water-retaining nature, and bulk density of soil (Zhou et al., 2020; Du et al., 2022a). Furthermore, tillage processes transfer MPs and other pollutants from the upper layer of soil to the bottom, and leaching can transport MPs to groundwater. MPs in soil not only restrict the functional diversity of soil microbes, but also influence nutrient absorption by plants and indirectly impacts plant development (Du et al., 2022b).

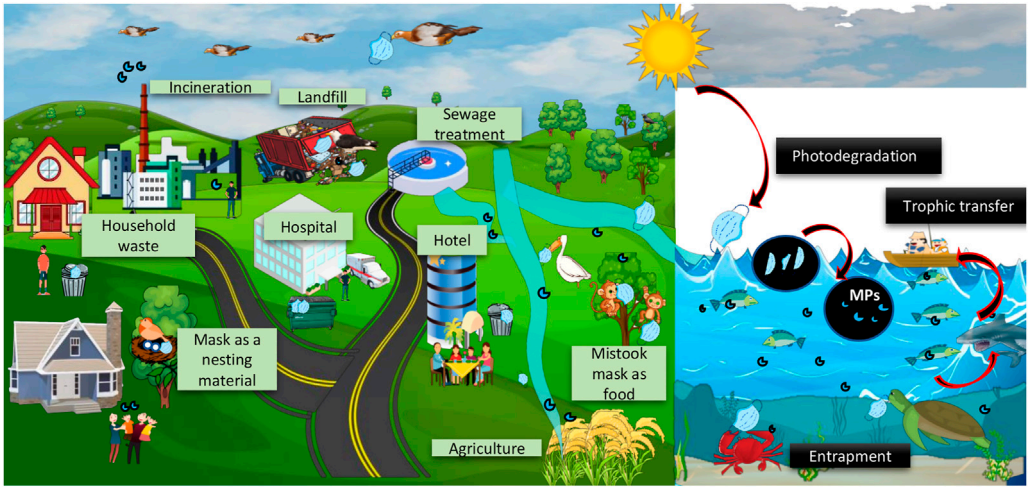

FIGURE 3
Source and impact of MPs associated with masks on terrestrial and aquatic systems.

Plant roots were found to be able to adsorb MPs, and these MPs penetrate into the plants *via* a crack-entry mechanism at root tips. Subsequently, transport from the root system to other tissues may happen primarily through a transpiration pull force (Li et al., 2020). Researchers also found that nano-sized microbeads may enter tobacco cells *via* endocytosis, implying that smaller plastics can enter the plant through rhizosphere adsorption (Su et al., 2019). As a consequence, through the trophic chain, these MPs will end up in the human body. For instance, MPs have been identified in feces, indicating human intake, and also their occurrence has recently been identified inside the human placenta (Schwabl et al., 2019; Ragusa et al., 2021).

3.2 Impact on the aquatic system

The inappropriate waste management of DFMs is highly menacing to aquatic systems as the rate of decomposition of plastics is comparable to be high in the soil. Temperature differences between these two distinct thermal habitats, partial submergence, water absorption capability, and other properties of the DFMs hampered the degradation of DFMs in aquatic habitats (Du et al., 2022a). DFM and other PPEs are often made of polymers such as latex, nitrile, or polyethylene that require the inclusion of additives such as softeners and stabilizers to improve physical qualities. When PPE kits are widely disseminated across the environment, these additives can be hazardous, and there is a significant risk of leaching into water bodies (Ray et al., 2022). DFMs can be disintegrated into MPs by undergoing weathering, corrosion, and aquatic immersion in natural conditions. Saliu et al. (2021) estimated the release of microfibres from DFM into the marine ecosystem when exposed to UV radiation. According to the findings, one DFM was exposed to 180 h of UV irradiation and severe mixing in artificial saltwater may release up to 173,000 fibers per day (Saliu et al., 2021). They have also discovered a similar chemical and morphological deterioration pattern in surgical masks found on Italian beaches, indicating that comparable processes may occur in the natural sea (Patricio Silva et al., 2021; Saliu et al., 2021). Furthermore, the hydrophobic nature of MPs aids in adsorbing organic compounds, resulting in bacterial colonization and microalgae development (Ray et al., 2022). This biofouling may contribute to the sinking out of immense plastic objects and attribute to a surge in marine pollution. Researchers found that, when NaCl concentration increases, the hydrodynamic diameter (D_h) of particulate plastic also increases which in turn causes PS MPs to aggregate. As a result, PS MPs are predicted to get aggregated in seawater (Cai et al., 2018). Due to microbial interactions, biofilm development can affect water quality by altering the microbiome and accelerating the rate of organic material decomposition. This leads to a reduction in dissolved oxygen (DO) content (Kirstein et al., 2019).

MPs are ubiquitous throughout the aquatic system and are mistaken as feed by many aquatic organisms. Certain properties of MPs such as micro-size, appealing hues, and great buoyancy make these tiny particles easily accessible to fishes and other organisms (Ray et al., 2022). For instance, MPs associated with masks and PPE have been reported in over 20% of marine crustaceans (Jeong et al., 2016). MPs are reported to be consumed by marine mammals such as whales and dolphins (Dharmaraj et al., 2021). Researchers have found that plastics were identified in the guts of 56% of aquatic birds. As a consequence, it was predicted that by 2050 identical

gastrointestinal issues are expected to infect 99.9% of birds (Aragaw, 2020). MPs of <1.5 μm in diameter can cause direct cell injury. Recent studies revealed that crabs intake MPs and it was found to get accumulated mostly in the hepatopancreas (Wang T. et al., 2021) and in 67% of shark samples, guts and digestive tracts showed a minimum of one MP (Parton et al., 2020). Aquatic megafauna and apex predators like whales, sharks, turtles, and mammals are at high risk of consuming whole masks (Fernandez and Anastasopoulou, 2019; Du et al., 2022a). MPs cannot be metabolized by organisms, and MPs containing biomolecules and aggregates can induce gastrointestinal or blockage issues. MPs can absorb water contaminants such as dye and hazardous compounds, which might be consumed by aquatic biota and impact the food chain (Binda et al., 2021). Plastic generated from DFM and other PPE can absorb organic and hazardous contaminants in aquatic systems, forming a hazardous film. This approach might harm aquatic biota that consumes plastic-based particles (Ray et al., 2022). These consumed MPs may influence reproduction, survival, and animal growth over time. Nanoplastics, together with genotoxins and oxidative stress have been found as a reservoir of neurotoxins in a wide range of aquatic biota, including corals (Chang et al., 2020). As a result of the bioaccumulation of these generated MPs in the primary food chain, human and animal health becomes intricate. For instance, a study on stool samples of adults and infants confirmed the presence of MPs. Surprisingly, MPs in infants are up to 20-fold higher than in adults (Zhang et al., 2021).

3.3 Impact on the atmosphere

The COVID-19 lockdown measures appear to minimize greenhouse gas emissions and enhance outdoor air quality. However, in the long run, the huge manufacturing of DFM and usage of other PPE causes a hidden problem of global greenhouse gas emissions. It was predicted that DFM has a greenhouse gas footprint of 0.059 kg carbon dioxide equivalents (CO_2-eq) (includes transport), whereas fabric masks have a footprint of 0.036 kg CO_2-eq/usage (includes rinsing) (Klemes et al., 2020). This implies that DFM use may have a tenfold greater impact on climate change than reusable masks (Yang et al., 2022). Globally, PP, PS, PVC, PE, and other airborne microplastics have been widely identified (Enyoh et al., 2019), which are the major components of DFMs. Airborne MPs flow through the atmosphere, accumulating in the air, water, and soil (Yang et al., 2022). The accumulation of MPs in the air expedites their threat of inhalation by humans. Furthermore, reports have also revealed that MPs in the DFM become a vital source of airborne MP pollution (Chen et al., 2021). Moreover, there is a high risk of MP inhalation, mainly when using low-quality DFM and repeated use of the same mask (Li et al., 2021).

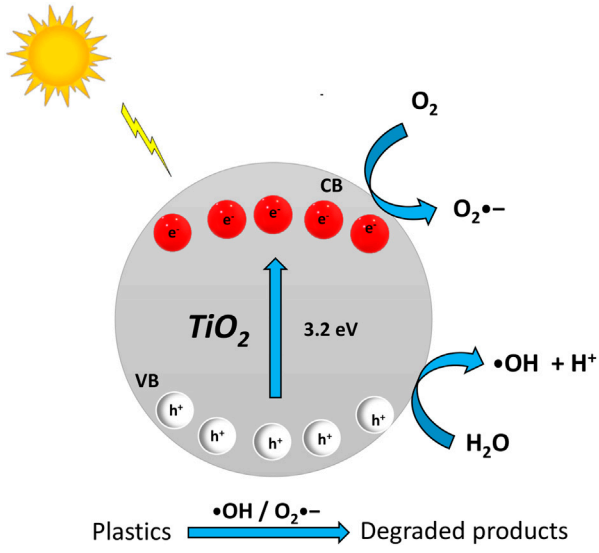

FIGURE 4
Schematic representation of TiO_2-mediated photocatalysis of plastics.

4 Role of photocatalyst in the degradation of plastics

Photocatalysis is one of the important methods in the advanced oxidation process (AOP). As this method utilizes solar energy, it implies minimal cost and an eco-friendly approach (Du et al., 2021). The underpinnings of photocatalytic degradation (PCD) are photocatalysts with strong redox potential. Semiconductor materials such as TiO_2, ZnO, WO_3, g-C_3N_4, CdS, SnO_2 ZrO_2, $BiVO_4$, and ZnS are employed as a photocatalysts in the PCD of organic contaminants (Lee and Li, 2021; Sharma et al., 2021). Once the absorbed energy of the photon (E) exceeds the semiconductor's band gap energy, electrons (e^-) in the valence band (VB) are transported to the conduction band (CB), and hence positive holes (h^+) are created in the VB, resulting in the dissociation of electron-hole pairs. Both species (e^- and h^+) react with O_2, H_2O, or OH, to form highly reactive oxygen species (ROS) (Nakata and Fujishima, 2012; Du et al., 2021). Such active species eventually disintegrate the organic polymers, causing polymeric chain breakage and even total mineralization (Sharma et al., 2021).

4.1 Titanium dioxide as photocatalyst

TiO_2 is an inevitable photocatalyst in plastic degradation. TiO_2 has a less bandgap energy between its VB and CB. When exposed to UV radiation, an electron in the VB gets excited and transfers to the CB (Figure 4). Electrons can migrate to or from the adsorbent, resulting in positively or negatively charged

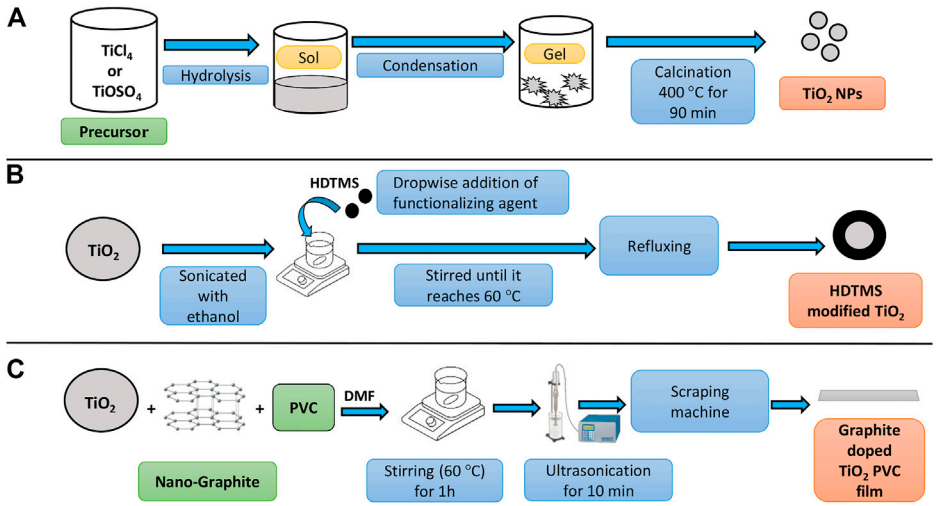

FIGURE 5
Flowchart illustration **(A)** Sol-gel synthesis of TiO_2, **(B)** Surface modification of HDTMS TiO_2 and **(C)** Graphite doped TiO_2 PVC film.

species. Anatase TiO_2 possesses a large bandgap of 3.2 eV, which relates to the excitation wavelength of 388 nm and enables it to absorb light in the UV range. Rutile TiO_2 has a bandgap of 3.0 eV and absorbs visible light with a wavelength of 410 nm (Martinez and Hammer, 2011; Li, 2020; Nabi et al., 2021). For PCD, anatase and rutile forms of TiO_2 are often used. Brookite forms are rarely utilized due to their unstable nature (Lee and Li, 2021). Iron oxide semiconductors are unstable because of their rapid photocathodic reactions and the resulting corrosive materials. Binary metal sulfide semiconductors like PbS, CdS, or CdSe are considered to be less stable due to their photoanodic corrosion nature and toxicity (Aziz et al., 2021). Though ZnO possesses a similar band gap to TiO_2, it is not much stable towards the pH. This instability leads to the $Zn(OH)_2$ precipitate formation on the surface of the particle, which results in the deactivation of the photocatalyst (Aziz et al., 2021). Furthermore, illumination of UV light upon ZnO leads to frequent photocorrosion (Barnes et al., 2013). In comparison with other photocatalysts, cost efficiency, mass production, doping, surface modifications, and well-established preparation processes are added advantages of TiO_2 in PCD (Zhang et al., 2019; Turkten and Bekbolet, 2020; Aziz et al., 2021).

4.2 Synthesis of Titanium dioxide-based photocatalytic nanomaterial/composites

4.2.1 Sol-gel

One of the most important processes in TiO_2 preparation is the sol-gel (also referred to as chemical solution deposition) method. The precursor and the reducer are the two main agents of the sol-gel method. (Li et al., 2010). Based on the type of titanium metal precursor, sol-gel synthesis of TiO_2 is classified into two types: 1) Aqueous-based approach (starting precursor: inorganic metal salt) and 2) alcohol-based approach (starting precursor: metal oxide). In the aqueous-based method, $TiCl_4$ and $TiOSO_4$ are essential precursors. The hydrolysis and condensation process may also be used to generate aqueous-based sol-gels as depicted in Figure 5A. Precipitation and peptization are two stages in the aqueous-based sol-gel process (Ullattil and Periyat, 2017). In the alcohol-based approach $Ti(OC_4H_9)_4$, $Ti(OC_3H_7)_4$, and $Ti(OC_2H_5)$ are the most prominent metal alkoxide precursors of TiO_2. There is a metal-oxygen link in these alkoxides, and because of the notable variation in electro-negativity between Ti and O, the bond turns highly polar and incredibly reactive. As a result of the addition of water, parallel hydrolysis and condensation events occur eventually leading to the development of a gel (Ullattil and Periyat, 2017). It was reported that TTIP (precursor) and ethanol (reducing agent) were employed in sol-gel TiO_2 preparation for the degradation of PE film. Researchers have also used $Ti(OBu)_4$ as a precursor and ethanol as a reducing agent. This method was used in the preparation of polypyrrole/TiO_2 nanocomposite for the degradation of PE (Thomas et al., 2013).

4.2.2 Surface modification

TiO_2 has been frequently modified to increase its efficiency and specificity. Modified TiO_2 characteristics may vary from pure TiO_2 in terms of charge separation, ease of TiO_2 particle separation, light, and pollution adsorption. Numerous studies have been conducted on TiO_2 modification, particularly for plastics deterioration. To prepare vitamin-C (VC) modified TiO_2, VC was dissolved in tetrahydrofuran (THF). The

mixture of n-TiO_2 powder and THF solution were mixed and ultrasonicated. VC–THF solutions were mixed with n-TiO_2 to obtain surface-modified n- TiO_2 with VC (Ariza-Tarazona et al., 2019).

Protein-based modified TiO_2 was synthesized using an extrapallial fluid of *Mytilus edulis*. This fluid acts as a pore-forming agent and N_2 precursor. This solution was mixed with titanium (IV) butoxide followed by mineralization and thermal treatment. This method was employed in the degradation of HDPE microplastics (Ariza-Tarazona et al., 2019).

Natural ore-based TiO_2 modifications were also reported. From leucoxene, ore rutile TiO_2 was obtained and prepared through a ball-milling planetary approach. It was mixed (20: 80) with (commercial) anatase with the help of a milling machine (Mekprasart et al., 2018). To synthesize surfactant-modified TiO_2, P25 TiO_2 was ground into fine powder. DI water and Triton X-100 (non-ionic surfactant) were added to the powder (dropwise manner) to obtain a paste. Ethanol was added to this paste (dropwise). This precursor solution was sonicated followed by string and used. This method aid in the degradation of PS microspheres and PE (Nabi et al., 2020).

Surface modification using a functionalizing agent is one of the important approaches in TiO_2 modification. TiO_2 NPs were added to ethanol and sonicated. Following the sonication, the dispersion was stirred until it reaches a favorable temperature (60°C). To this dispersion hexadecyltrimethoxysilane (HDTMS-functionalizing agent) was added (dropwise) as per the required degree of functionalization. Further, the temperature was enhanced followed by refluxing. Finally, the hexadecyltrimethoxysilane-modified TiO_2 (resultant material) was rinsed and dried as depicted in Figure 5B (Alvarado et al., 2016).

$Fe(St)_3$ modified TiO_2 was prepared by the addition of TiO_2 and ferric stearate to tetrahydrofuran. This combination was mixed with KH550 silicone by ultrasonication. This suspension was coated with PS to obtain the plastic-photocatalyst combination by stirring (12 h). The resulting material was placed on a glass stick and dried. This approach was employed in the degradation of PS (Fa et al., 2013).

4.2.3 Doping

TiO_2 doping is a process carried out to enhance the reactivity of TiO_2 and results in additional energy to the band structure that can have an effective role in the transmission of charges to the surface (Lee and Li, 2021). To fabricate Graphite doped (GrD) TiO_2, PVC plastics were used. Graphite, TiO_2, N, N dimethylformamide (DMF), and 2 g PVC were stirred (1 h; 60°C) followed by the ultrasonication of 10 min. Using the scraping instrument, the resulting material was uniformly spread onto a substrate. Finally, the film-generating plate was removed and soaked in DI water to obtain GrD TiO_2 PVC film as depicted in Figure 5C (Peerakiatkhajohn et al., 2011; Nabi et al., 2021).

To enhance the activity of TiO_2, transition metals were employed in doping. Titanium n-butoxide, ethanol, acetylacetone, nitric acid, and DI water were mixed and stirred at room temperature. Meanwhile, silver nitrate was produced by mixing $AgNO_3$ with ethanol. The silver nitrate solution was then stirred into TiO_2 and refluxed (8 h; 80°C) (Peerakiatkhajohn et al., 2011; Lee and Li, 2021).

TiO_2 multi-walled carbon nanotubes (MWCNT) composites were also adopted in plastic degradation. MWCNT was treated with the acid vapor method. An MWCNT was calcined (450°C; 1 h) to eliminate the amorphous carbon. After thermal treatment, an MWCNT was put onto a silicon griddle in a Teflon (HNO_3 was already present at the bottom). The resulting material was rinsed and dried. Then, titanium butoxide was mixed with ethanol and marked as A. Processed MW carbon was dissolved in ethanol through sonication, after the additament of acetic acid, with pH = 2-3, and termed B. Mixture "A" was added to "B" and mixed for about 15 min. This solution was stirred in a water bath (60°C) before being shifted to an autoclave and heated at 180°C. TiO_2 particles develop over the CNT during thermal treatment, resulting in grey precipitates. Followed by the ethanol wash, the end product was dried (6 h at 90°C). This approach was employed in the degradation of PE (An et al., 2014).

4.3 Titanium dioxide assisted photocatalytic degradation of microplastics

The TiO_2-embedment approach was used to degrade plastics such as PE, PP, PVC, PS, and PS polymers under diverse parameters (Fa et al., 2013; Nabi et al., 2020; Hamd et al., 2022). The PCD of PS plastics was carried out under UV irradiation using PS-TiO_2 composite. Results revealed that better weight reduction (85%) was observed in PS-TiO_2 composite than in pure PS film (65%). This was supported by the SEM results of PS-TiO_2 samples as it has more voids compared to pure PS film. Doping also plays a crucial part in plastic degradation. Undoped and metal (Iron, silver, and Iron/silver-mix) doped TiO_2 NPs were used to compare the PCD of PE films. The Fe/Ag doped TiO_2 exhibited a maximum weight reduction of 14.43% (UV irradiation). As the irradiation time increases the rate of weight reduction also increased. SEM analysis revealed the formation of voids in the PE matrix. It is suggested that ROS generation on the surface of TiO_2 is attributed to the degradation of the PE matrix (Figures 6A,B) (Asghar et al., 2011). Similarly, copper phthalocyanine-modified TiO_2 showed better PE degradation than bare TiO_2 (Zhao et al., 2008).

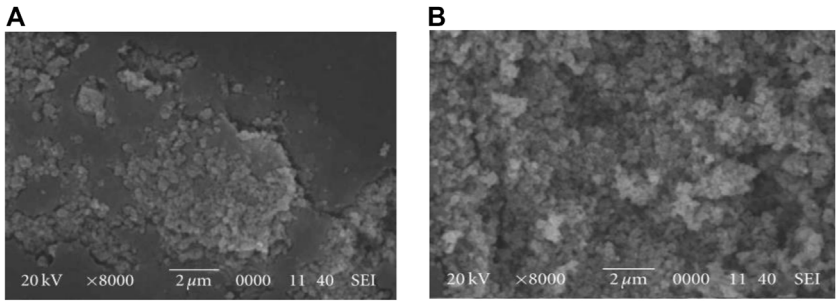

FIGURE 6
SEM images of **(A)** PE-TiO_2 film after irradiation and **(B)** PE-Fe/Ag mix doped TiO_2 film after irradiation (Reprinted from Asghar et al., 2011).

TABLE 1 Applications of TiO_2-based nanomaterials in photocatalytic degradation of plastics.

MP degraded	Nanomaterial used (catalyst)	Result/degradation efficiency (DE)	Irradiation conditions	References
HDPE	C, N-TiO_2	Mass reduction: 75%	27 W light; Duration: 50 h	Ariza-Tarazona et al. (2020)
PE	Triton X-100-based TiO_2	Fully decomposed	254 nm (UV light)	Nabi et al. (2020)
PE	CuPc modified TiO_2	DE: 36%	Solar light; Duration: 160 h	Zhao et al. (2008)
PE	Ag/TiO_2/RGO	DE: 76%	UV light	Fadli et al. (2021)
PE	Nano-composite Ag/TiO_2	DE: 100%	UV light	Maulana et al. (2021)
PE	Polyacrylamide grafted TiO_2	DE: 39.85%	UV light; Duration: 520 h	Liang et al. (2013)
PE film	Ag-doped TiO_2	DE: 14.28%	UV light; Duration: 300 h	Asghar et al. (2011)
PVC	Vitamin C–TiO_2	Mass reduction: 71%	UV light; Duration: 216 h	Yang et al. (2010)
PE film	TiO_2 multi-walled CNT	DE: 35%	UV light; Duration: 180 h	An et al. (2014)
HDPE, LDPE	N-TiO_2	Mass reduction: 4.65%, 1.8% respectively	Duration: 50 h	Rodríguez-Narvaez et al. (2021)
HDPE	N-TiO_2	Mass reduction: 6%	27 W light; Duration: 20 h	Ariza-Tarazona et al. (2019)
PS	Anodized TiO_2	DE: 23.5%	UV light	Domínguez-Jaimes et al. (2021)
PS (400 nm)	Ethanol-based TiO_2	DE: 91.04%	UV light (365 nm); Duration: 12 h	Ariza-Tarazona et al. (2019)
PS	CuPc-TiO_2	DE: 6.9%	Fluorescent lamp; Duration: 250 h	Shang et al. (2003a)
PS	TiO_2/Fe(St)3	Reduction in molecular weight by 79.49%	UV light; Duration: 480 h	Fa et al. (2013)
PS	TiO_2	DE: 22.5%	UV light; Duration: 150 h	Shang et al. (2003b)
PS (400 nm)	Triton X-100-based TiO_2	DE: 98.40%	UV light (365 nm); Duration: 12 h	Nabi et al. (2020)
PS	FePc-TiO_2	DE: 35%	Sunlight; Duration: 250 h	Fa et al. (2008)
PS	Grafted TiO_2	DE: 29%	UV light; Duration: 300 h	Zan et al. (2004)

Reduced graphene oxide coated TiO_2 (TiO_2-rGO) is one of the important catalysts in the degradation of PP. This is mainly due to the Ti-O-C link, rGO extends TiO_2's absorption range to the visible area. Furthermore, 2D π-conjugation of rGO helps in the reduction of the recombination rate (Lee and Li, 2021). FT-IR indicates the presence of a carbonyl group with an increased carbonyl index, resulting in the more effective photooxidation of PP by TiO_2-rGO nanocomposite than TiO_2 NPs. Studies have revealed the PCD of PVC films under the air/nitrogen atmosphere. There was a 27% degradation in presence of air and no degradation was observed under a nitrogen atmosphere. This study implies the importance of O_2 in photocatalysis (Cho and Choi, 2001). Applications of TiO_2-based nanomaterials in PCD of MPs are depicted in Table 1.

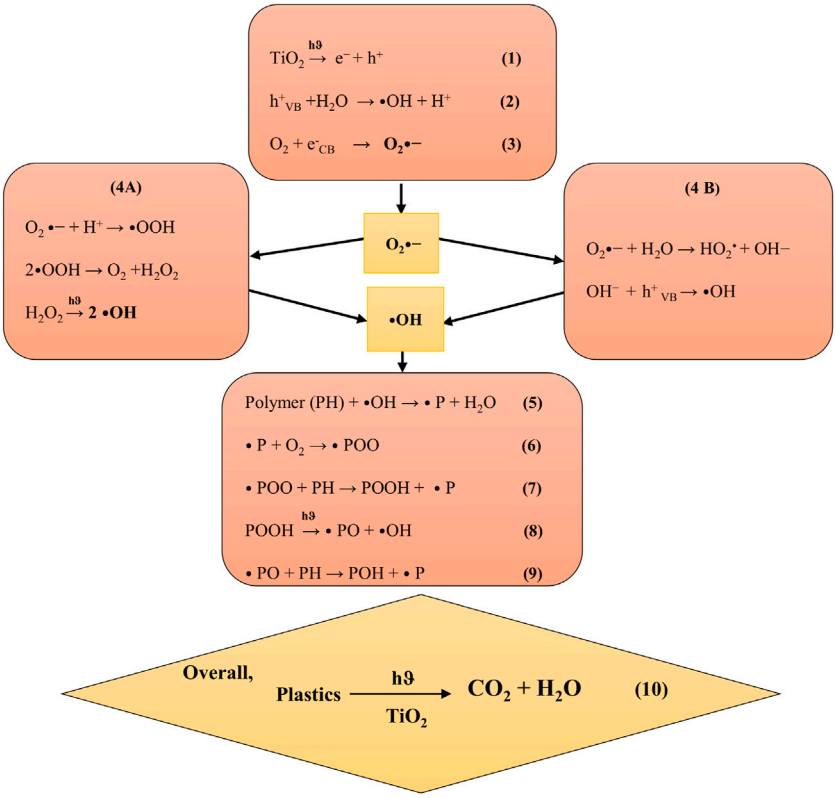

FIGURE 7
Mechanism of photocatalytic degradation mediated by TiO_2.

4.3.1 Mechanism of photodegradation

When the photocatalyst absorbs UV light (with energy equal to or greater than its bandgap of TiO_2), the electrons in VB get excited to CB. This forms holes in VB and subsequent electron-hole recombination (Eq. 1). Water interacts with the holes (present in VB) and generates hydroxyl radicals (•OH) (Eq. 2). The photocatalytic breakdown of plastics is significantly influenced by O_2 molecules. Superoxide anions are generated by the interaction of O_2 molecules (on the TiO_2 surface) and free electrons (Eq. 3) (Ma et al., 2020). These superoxide anions also form •OH, which plays a key role in plastic degradation (Lee and Li, 2021). The formation of •OH can proceed in two ways, 1) The superoxide anion gets protonated to give hydrogen peroxide, which is subsequently dissociated by light energy to form •OH (Eq. 4A). 2) Superoxide anion combines with water to give HO_2• and OH^-. This OH^- occupies holes to form •OH (Eq. 4B). This generated •OH degrades the plastics into CO_2 and water. (Eqs. 5–10) (Chen et al., 2019; Lee and Li, 2021; Nabi et al., 2021). The mechanism of TiO_2-based photocatalytic degradation of plastics is given in Figure 7.

4.3.2 Factors influencing the photocatalysis of plastics

The properties of photocatalysts and plastics play a prominent role in plastic degradation. Mainly, the type of photocatalyst and the physiochemical properties of the plastics are some of the critical factors in plastic degradation. As the size of the plastic decreases, the more will be the specific surface area. This leads to enhanced reactivity with O_2 or photons, which enhances smaller (sized) plastic degradation than the larger ones (Sharma et al., 2021). TiO_2-based nanomaterials play a vital role as a photocatalyst in the degradation of MPs. This is mostly owing to its high organic pollutant oxidation ability (Yuan et al., 2017). Furthermore, environmental factors like light, dampness, temperature, O_2 concentration, and pH affect plastic deterioration.

4.3.2.1 Plastics and the photocatalyst used

The size of the plastic has a considerable influence on photodegradation efficiency. The lesser the structure

complexity, the more will be the deterioration. (Song et al., 2017; Uheida et al., 2021). For instance, by using TiO_2, researchers have studied the photodegradation of PE and PS. Due to its less complicated structure, the rate of degradation of PE was comparably high to that of PS (Zhao et al., 2008). Crystal type is also noted to be an influencing factor. The anatase phase in TiO_2 (when doped with Mo and W) determines the PS degradation efficiency. Reports also suggest that doping Mn and Cr with TiO_2 reduces the rate of degradation of PS film (Zhao et al., 2007; Ge et al., 2022). The quantity of photocatalysts used can affect the degradation efficiency of plastics. For instance, the rate of degradation of PE- TiO_2 film increased as the concentration of TiO_2 increased (Ge et al., 2022). The type of catalyst used is an important factor that influences plastic degradation. Ariza-Tarazona et al. (2019) used two distinct semiconductor photocatalysts relying on N-TiO_2 to evaluate the breakdown of HDPE MPs obtained from a cosmetic scrub. The first catalyst was obtained from *Mytilus edulis* (green synthesis). The second catalyst was fabricated by utilizing urea and a tri-block copolymer. The photocatalytic technique proceeded under visible light for 20 h, and the protein-derived catalyst showed a strong capability to assist photodegradation in aqueous as well as solid media. The protein-derived catalyst degraded HDPE at a rate constant of 38.2 ± 3.7 (aqueous phase) and 12.2 ± 0.8 (solid phase). Mass loss was 6.4% in the aqueous phase and 1.1% in the solid phase (Ariza-Tarazona et al., 2019). Degradation of PE (photocatalytic) was studied between PE- TiO_2 composite and pure PE (photolytic reaction). The degradation was higher in PE- TiO_2 composite than pure PE. This shows the efficiency of Photocatalyst in the plastic degradation (Zhao et al., 2007).

4.3.2.2 Light

The light source can influence the result of plastic degradation to a great extent. Solar irradiation contributes inevitably to the degradation process. Mainly, IR rays (>700 nm have enhanced thermal oxidation, UV (<400 nm) direct photodegradation, and visible rays (400–700 nm) promote degradation through heat (Thomas and Sandhyarani, 2013). Under UV irradiation, most plastic particles/films degrade partially. This means that the active oxygen species produced (when exposed to visible light) are incapable of triggering chain cleavage and subsequent oxidation processes. To address this, researchers have used semiconductors as photocatalysts to improve photodegradation (Du et al., 2021). The degradation of PE in the presence of sunlight using polypyrrole (PPy)/TiO_2 nanocomposite as a photocatalyst, which was synthesized using emulsion polymerization and sol-gel processes. It was noted that exposing the PE material to daylight for 240 h lowered its molecular weight up to 35.4% and 54.4% respectively (Li et al., 2010). The PCD of LDPE was achieved with the help of N- TiO_2. The degradation was carried out under solar irradiation for 200 h. Results after the irradiation revealed a significant weight of 68% (Thomas and Sandhyarani, 2013).

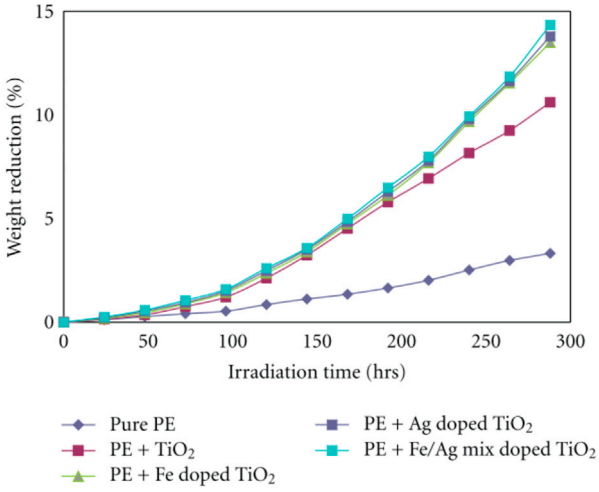

FIGURE 8
Effect of UV irradiation and irradiation time on the degradation of PE films (Reprinted from Asghar et al., 2011).

UV radiation promotes photooxidative degradation followed by polymer breakage mediated by photons and the produced free radicals degrade the plastics (Verma et al., 2017). Studies have also revealed the significance of UV irradiation and the irradiation time in the degradation of plastics. Reports have revealed the importance of irradiation time in the PCD of PE films using undoped and metal (Fe, Ag) doped TiO_2. As the irradiation time increased, the weight reduction percentage of PE films has also increased. The maximum weight reduction was observed at 300 h (Asghar et al., 2011). Figure 8 depicts the significance of irradiation time in PE film degradation.

PE-goethite films were irradiated in UV light with two different intensities (2 mW/cm^2 and 1 mW/cm^2). The greater intensity leads to a better weight loss of 24% higher than the films treated with lesser intensity. This shift in light intensity enables changes in the electron-hole pair, such as the rate of recombination and separation on the photocatalyst surface (Li et al., 2010).

4.3.2.3 Other factors

The significance of humidity in PCD of PE was observed in a study. The degradation experiment was performed using n-TiO_2 without a proper source of humidity (at room humidity). This condition leads to the termination of the reaction. Once the humidity in the ambient atmosphere was allowed to pass through the reaction chamber, the reaction proceeded (Ariza-Tarazona et al., 2019). Reports also suggest the importance of O_2 in PCD. The rate of weight loss was examined between the PVC- TiO_2 film in anoxic and oxic conditions. The quantity of weight loss was greater in the air, and the period of deterioration was similarly shorter. This is mainly due to the production of ROS

due to the interaction of O_2 with the CB electrons (Cho and Choi, 2001). The optimum pH of is one the important factor in the PCD. In the degradation of HDPE by C, N–TiO_2, the interaction among MPs and colloidal NPS was promoted due to the lower pH. In addition, reduced temperature (0°C) (facilitates an increased surface area) also favors the degradation of HDPE (Ariza-Tarazona et al., 2020).

5 Challenges

Despite the numerous benefits of the photocatalytic degradation of MPs (plastics), there are still certain challenges that must be addressed. One of the important limitations is the specificity of photocatalysts i.e., photocatalysts are often suited for degrading only a particular type of plastic. Furthermore, photocatalysts cannot selectively target the plastic's reactive sites (functional groups or defects) (Ge et al., 2022). In the environment, MPs plastics are found to be combined with other co-pollutants. Degradation of MPs along with the co-pollutants using PCD might be a challenging factor. Photocatalysis is a surface reaction, photodegradation may get impeded if light irradiation is hampered by non-transparent materials (contaminants). Additionally, certain TiO_2-based photocatalysts function better in the UV band but not in visible light conditions. Owing to their versatility, TiO_2-based photocatalysts are extensively employed in the photocatalytic degradation process. Reports suggest that the anatase form TiO_2 is more toxic than the rutile form (Shabbir et al., 2021). This increased toxicity of the anatase form is attributed to the smaller particle size and the increased surface area (Shabbir et al., 2021). Studies have also revealed the toxicity of TiO_2 toward the algae (primary producer) and its possibility to affect the (algae-crustacean) food chain (Bhuvaneshwari et al., 2018). Extensive investigations on the ecotoxicological assessment of TiO_2 NPs are essential to analyze the toxicity of TiO_2 NPs towards the environment. Additionally, analyzing the toxicity of the PCD intermediates would be a strenuous process.

6 Conclusion and future perspectives

Plastic pollution has become a worldwide issue due to its negative impact on human health and the environment. Furthermore, the COVID-19 pandemic has exacerbated this problem, owing to the widespread usage of PPE such as masks, gloves, and other plastic-based equipment. As these plastics get degraded into microplastics (under natural conditions), the chance of efficient mitigation is a conundrum. PCD could be a possible eco-friendly approach as it utilizes sunlight as an energy source.

Recent advancements in novel technologies and the production of innovative photocatalytic materials ameliorate plastic degradation. Future investigations on the toxicity of the intermediates that are released and the reusability of the photocatalyst are needed. In most of the PCD processes, CO_2 will be generated as an end product. Even though it is less toxic than plastic waste, undoubtedly addressing these issues is necessary. Extensive investigations on the ecotoxicological assessment of TiO_2 NPs are essential to analyze their toxicity towards the environment. To overcome these ecotoxicological issues, investigations on the usage of green nanomaterials as photocatalysts will be an eco-friendly approach towards plastic degradation. Green synthesis of TiO_2 is a sustainable process that offers significant advantages over chemical and physical approaches such as non-toxicity, low cost, biocompatibility, and high efficiency. Future studies can focus on developing more visible-light-driven TiO_2-based photocatalytic materials. As the MPs are found to be combined with other co-pollutants in the environment, suitable approaches for the mitigation of combined pollution should be taken into consideration.

Author contributions

CM: Writing- original draft, review, and editing. AM: Conceptualization, review, editing and supervision.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Using nanomaterials to address SARS-CoV-2 variants through development of vaccines and therapeutics

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Nanomaterials have played a significant role in effectively combating the global SARS-CoV-2 pandemic that began in December 2019 through the development of vaccines as well as antiviral therapies. These versatile, tunable materials can interact and deliver a broad range of biologically relevant molecules for preventing COVID-19 infection, generating immunity against COVID-19, and treating infected patients. Application of these nanomaterials and nanotechnologies can further be investigated in conjunction with disease models of COVID-19 and this holds immense potential for accelerating vaccine or therapeutic process development further encouraging the elimination of animal model use during preclinical stages. This review examines the existing literature on COVID-19 related nanomaterial applications, including perspective on nanotechnology-based vaccines and therapeutics, and discusses how these tools can be adapted to address new SARS-CoV-2 variants of concern. We also analyze the limitations of current nanomaterial approaches to managing COVID-19 and its variants alongside the challenges posed when implementing this technology. We end by providing avenues for future developments specific to disease modelling in this ever-evolving field.

KEYWORDS

nanotechnology, COVID-19, nanomaterials, vaccine development, antiviral therapeutics, disease-modelling, lipid nanoparticles, inorganic nanoparticles

Introduction

The COVID-19 pandemic, a historical event that will impact several generations, started with a pneumonia outbreak in Wuhan, China in January 2020. A new virus (SARS-CoV-2) was discovered that infected human lung epithelial cells. The global spread of SARS-CoV-2, along with the death of thousands, led to the World Health Organization to declare a global pandemic on 12 March 2020 (Ciotti et al., 2020). The ongoing spread of SARS-CoV-2 and its variants has imposed a significant burden on people around the world. Governments have implemented curfews, lockdown, physical distancing, and quarantining to prevent the disease from spreading (Miyamura, 2021). The requirements for social distancing along with the enforcement of lockdowns had a major impact on many businesses and jobs, leading to an increase in unemployment and other devastating effects on the global economy (Kawohl and Nordt, 2020). Moreover, the COVID-19 pandemic also led to psychological struggles, including high amounts of psychological stress as well as anxiety due to lack of social interactions (Banerjee, 2020). Additionally, the fear of infection causes stress during large gatherings, including events like weddings and funerals.

It is hypothesized that the origins of the disease resulted in infected animals sold in wet markets due to similarities of the SARS-CoV-2 RNA sequences to known coronaviruses found in bats (Morens et al., 2020). SARS-CoV-2 belongs to the genera Betacoronavirus, and it consists of spherical enveloped particles ranging in diameter from 70 to 100 nm covered in club-shaped glycoproteins (Mousavizadeh and Ghasemi, 2021). The particles contain a single-stranded RNA genome (Mousavizadeh and Ghasemi, 2021). SARS-CoV-2 has a spike protein containing two subunits: S1 and S2 (Choi et al., 2021). S1 enables the virus to attach to host cell surface receptors while S2 promotes the fusion of host cells with viral membranes (Choi et al., 2021) as shown in Figure 1 (Belete, 2020). The spike protein also to angiotensin-converting enzyme 2 (ACE2) receptors which enables the take up of viruses into host cells (Nugent, 2022). Because human host cells contain large amounts of ACE2, type II alveolar epithelial cellsare thought to be the main target for infection (Mondal et al., 2022). The virus is transmitted through inhalation or contact with droplets containing viral particles, and the incubation period ranges within 2–14 days of infection (Choi et al., 2021). Symptoms of SARS-CoV-2 range from mild to severe and include fever, cough, shortness of breath, vomiting, diarrhea, and abdominal pain (Ciotti et al., 2020). Other reported symptoms include severe pneumonia, acute respiratory distress syndrome (ARDS), and organ failure (Choi et al., 2021). However, these symptoms are progressively changing as new variants arise. For example, the delta variant has a higher risk for hospitalization compared to the alpha variant (Shiehzadegan et al., 2021).

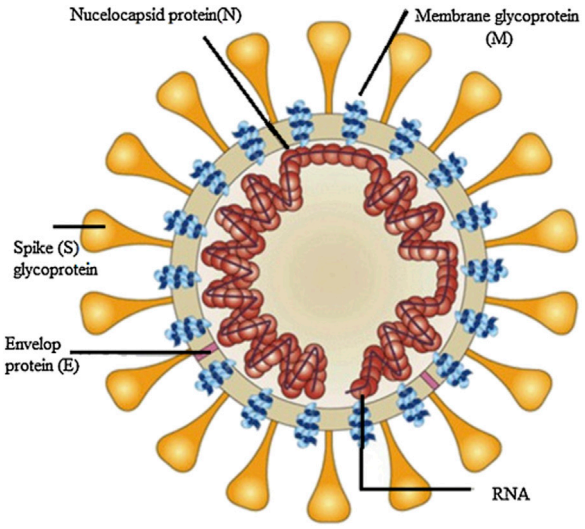

FIGURE 1
Simple Illustration of Coronavirus structure sourced from A review on Promising vaccine development progress for COVID-19 disease adapted from (Belete, 2020). Reproduced with permission of Elsevier as this work is part of their open access COVID-19 resource centre.

New variants of concern (VOC) have emerged during the pandemic as the virus has mutated. A variant of concern contains mutations that can increase transmission rates, worsen symptoms from those caused by the original virus, and/or provide viral immunity from treatments and preventative methods such as vaccines (Otto et al., 2021). Currently, these VOCs include the Alpha, Beta, Gamma, Delta, and Omicron variants (Zeng et al., 2022). These variants differ from each other due to differences in their genome sequence encoding for variations in spike protein structure (Hadj Hassine, 2022a). These subtle yet significant differences in spike protein led to changes in viral properties, such as, how contagious the variants are (transmissibility) and the types of symptoms, as well as, the severity of symptoms that can be experienced by infected individuals. Changes in viral properties can lead to issues in vaccine efficacy, as vaccines formulated for one variant may not be able to effectively mediate symptoms induced by another version of the viral pathogen.

Novel forms of nanotechnology are currently being used to treat and prevent the infection of the virus, controlling the progression of SARS-CoV-2 and easing the burdens of the pandemic. Nanotechnology uses materials with dimensions ranging from 1 to 100 nm (Hulla et al., 2015), including naturally produced particles like proteins, and synthetically produced molecules like metal nanoparticles (Mcneil, 2011). The small size, higher solubility, and multifunctionality of nanomaterials provides benefits when addressing medical issues (Mcneil, 2005). Many nanomaterials, such as quantum dots, metal nanoparticles, and nanowires (Gao and Xu, 2009), can be formulated to induce specific effects in human cells and

tissues (Chakraborty et al., 2011). They can also be functionalized with different ligands for applications in drug delivery, imaging, and therapeutics. The versatile nature of nanotechnology has significant promise for managing SARS-CoV-2 through diagnostics, vaccines, and therapeutics. This review will discuss properties of nanomaterials currently being used and or are under consideration for managing the SARS-CoV-2 virus and their future application as an important tool for managing variants of concern.

2 Nanotechnology advancements in developing vaccines

In recent years, advancements in the field of nanotechnology have led to vaccines that are used to protect against current and future variants of COVID-19. Both traditional and mRNA vaccines illicit consistent immune responses towards fighting infection despite the varying mechanisms to how these responses are induced (Bullock, 2021). They both activate the adaptive immune system consisting of B-cells, antibodies, and T-cells. The adaptive immune system produces antibodies which neutralize the virus. The information of how to make these antibodies to fight against future infection is stored in memory B and T cells. This memory is what gives a vaccine recipient lasting immunity. The difference between traditional vaccines and mRNA vaccines is that traditional vaccines typically use a weakened virus or the antigen of a virus as a whole (Pardi et al., 2018) to generate immunity, whereas mRNA vaccines generate the same immunity by using the host cell machinery to synthesize the antigen of a virus encoded in a delivered mRNA strand. Here, we discuss traditional vaccine types and the innovation of mRNA vaccines against COVID-19. This section highlights nanotechnology-based formulations, specifically lipid nanoparticles (LNPs), their composition and manufacturing, and the nanomaterial-based ingredients playing a role in delivering mRNA cargo used to address SARS-CoV-2 and its variants of concern.

2.1 Current non-mRNA vaccines for SARS-CoV-2

COVID-19 vaccines work to prevent or lessen the symptoms of an infection (Bonanni and Santos, 2011) by first eliciting an innate immune response by exposing the immune system to the COVID-19 spike protein so that if the pathogen is encountered, the presence of SARS-CoV-2 antibodies will enable a stronger immune response (Mathieu et al., 2021; Sette and Crotty, 2021). Although mRNA vaccines are widely used, non-mRNA vaccines, which include viral vector vaccines, protein subunit vaccines, and whole pathogen vaccines, are also present in the market shown to induce immunity against COVID-19 through different mechanisms.

Viral vector vaccines consist of engineered, chemically weakened viruses with SARS-CoV-2 genes. They infect immune cells to induce the production of COVID-19 proteins or display COVID-19 proteins on their surface (Samaranayake et al., 2021). Viral vector vaccines can be replicating (i.e. can make a new viral particle) or non-replicating (i.e. cannot make a new viral particle) (Samaranayake et al., 2021). Non-replicating viral vector vaccine technology has been used by Oxford-AstraZeneca to create the Vaxzevria COVID-19 vaccine (ChAdOx1 nCoV-19) and by Janssen to create the Janssen Jcovden COVID-19 Vaccine (Ad26.COV2.S) (Mathieu et al., 2021; Voysey et al., 2021; Andrews et al., 2022).

Subunit vaccines contain SARS-CoV-2 whole proteins or their protein fragments (Samaranayake et al., 2021). The Novavax Nuvaxovid COVID-19 vaccine (NVX-CoV2373) is an approved and effective protein subunit vaccine which carries a recombinant SARS-CoV-2 spike protein (Keech et al., 2020). While Novavax vaccines targeting the Beta and Delta variants of COVID-19 are in clinical trials, the production of COVID-19 variant-specific protein-subunit vaccines has been delayed compared with mRNA vaccines due to differences in vaccine development procedures (Lee and Kim, 2022).

Virus-like particle vaccines are a whole pathogen type of vaccine that mimics the structure of the SARS-CoV-2 virus that do not contain genetic material and are not infectious (Ndwandwe and Wiysonge, 2021). In Canada, the Medicago Covifenz COVID-19 vaccine is an approved plant-based virus-like particle vaccine (Hager et al., 2022). This coronavirus-like particle vaccine utilizes *Nicotiana benthamiana* plants to produce virus-like particles of a modified spike glycoprotein from SARS-CoV-2 strain hCoV-19/USA/CA2/2020 (Stander et al., 2022). Although there are none approved in Canada, whole-pathogen vaccines, such as live attenuated vaccines and inactivated vaccines, which utilize a weakened form of the virus to stimulate immune responses, area also in development for the SARS-CoV-2 virus (Ndwandwe and Wiysonge, 2021).

The utilization of various types of COVID-19 vaccines has been met with various levels of success as the type of vaccine influences the degree of significant reduction of infectious symptoms, transmissibility, and mortality outcomes at a global scale (Baden et al., 2021; Mathieu et al., 2021; Voysey et al., 2021; Andrews et al., 2022). Highly successful vaccines are characterized based on efficacy and are dependent on two factors: potency and administration. In order to assess vaccine efficacy, the seroconversion must be determined. It is a quantitative measure of antibody persistence which defines the level of immune response (Orenstein et al., 1985). In a 2021 review by Yan et al. (2021), vaccine trials and obtained seroconversion rates of mRNA-based vaccines as well as non-mRNA-based vaccines were summarized. While Pfizer/BioNTech's BNT162b1/b2 mRNA lipid nanoparticle vaccine demonstrated a dose-dependent antibody response with no serious adverse events, Moderna's mRNA-1273 demonstrated

100% seroconversion rates by day 15. On the other hand, traditional viral vaccines like Oxford-AstraZeneca's ChAdOx1 nCoV-19 had shown to induce antibody titres five times more when comparing booster doses to single-dose groups with noted serious adverse events. Protein subunit vaccines like Novavax's NVX-CoV2373 were demonstrated to have antibody responses such as neutralization levels and IgG GMT presence exceeding normal serum levels.

A metanalysis of vaccine efficacy in protecting against severe disease caused by COVID-19 reveals that, in adults, two doses of the Vaxzevria COVID-19 vaccine has 74% efficacy, a single dose of the Janssen Jcovden COVID-19 vaccine has 66% efficacy, two doses of the Novavax Nuvaxovid COVID-19 vaccine has 90% efficacy, and two doses of the Medicago Covifenz COVID-19 vaccine has 71% efficacy (Government of Canada 2020b; Government of Canada 2020c; Government of Canada 2020e; Government of Canada, 2022a). Two doses of the Pfizer-BioNTech Comirnaty mRNA COVID-19 vaccine possessed 95% efficacy in individuals 16 years and older and two doses of the Moderna Spikevax mRNA vaccine exhibited 94.1% efficacy in adults (Government of Canada 2022d; Government of Canada, 2022f)Of these COVID-19 vaccines, the mRNA-based vaccines, Pfizer-BioNTech Comirnaty and Moderna Spikevax, had greater efficacy than approved traditional COVID-19 vaccines. Given this data, mRNA nanoparticle-based vaccines have demonstrated to show higher success in fighting against viral pathogens like SARS-CoV-2.

2.2 mRNA-lipid nanoparticle technologies

Two nanoparticle-based vaccines for COVID-19 have been approved for use by Health Canada after their determination as safe and effective: the Pfizer-BioNTech Comirnaty COVID-19 vaccine (BNT162b2 mRNA) (Polack et al., 2020) and Moderna Spikevax COVID-19 vaccine (mRNA-1273 SARS-CoV-2) (Baden et al., 2021). These vaccines utilize LNP technology to deliver an mRNA transcript intramuscularly for the *in vivo* translation and protein synthesis of the COVID-19 spike glycoprotein (Kowalzik et al., 2021).

When delivered without a carrier, the uptake of mRNA by cells is low and considered unsuitable for therapeutic use due to the quick degradation rate of the mRNA in circulation (Kowalzik et al., 2021). Naked mRNA is negatively charged, large, and unstable, making its passive diffusion across the cell membrane challenging (Sahin et al., 2014). LNPs have arisen as powerful tools for the delivery of mRNA as they address these issues. LNPs envelop mRNA and act to protect mRNA from degradation and deliver it to the cytosol of target cells (Melamed et al., 2022).

2.2.1 Lipid nanoparticle composition and function for mRNA delivery

The LNPs used in Pfizer and Moderna's mRNA COVID-19 vaccines have four primary components: ionisable cationic lipids, helper lipids including neutral phospholipids, cholesterol, and polyethylene-glycol (PEG)-lipids (Figure 2) (Evers et al., 2018; Zhi et al., 2018; Albertsen et al., 2022). The most important part of these components is the ionizable lipids as they promote particle uptake by the desired cell population. LNPs loaded with mRNA have a core-shell structure (Albertsen et al., 2022; Pantelić et al., 2022). The nanoparticles consist of a cationic lipid shell containing the mRNA. The ionizable lipids, helper lipids, cholesterol and PEG-lipids form a lipid shell around these core molecules (Alameh et al., 2021; Hassine, 2022). Cationic ionizable lipids have a positively charged head group that make up the bulk of the LNP bilayer (Zhi et al., 2018). The cationic ionizable lipids used in LNPs generally have a pKa just below neutral pH resulting in a positive charge at acidic pH and a neutral charge at a pH above the pKa. The lipids and SM-102 and Alc-0315, used by Pfizer and Moderna, have pKas of 6.09 and 6.75 respectively.

Production of the LNPs occurs at an acidic pH of around 4 (Cheng and Lee, 2016; Kulkarni et al., 2018). The positive charge of these lipids interacts with negatively charged nucleic acids through electrostatic interactions, increasing the encapsulation efficiency of the mRNA (Cheng and Lee, 2016; Evers et al., 2018; Kulkarni et al., 2018; Zhi et al., 2018). This interaction leads to the formation of a lipid bilayer, sandwiching condensed nucleic acids. However, at physiological pH (7.4) the ionizable cationic lipids have a neutral charge (Evers et al., 2018; Schlich et al., 2021). This effect protects against clearance of the LNPs by the immune system which would occur if permanently changed LNPs were used (Schlich et al., 2021). This property also avoids the uptake of negatively charged molecules by the LNP if a permanently charged lipid were to be used (Evers et al., 2018).

An advantage of using ionizable cationic lipids is that they promote endosomal escape within the target cells (Evers et al., 2018; Schlich et al., 2021). Escape occurs because the pH of the endosome is more acidic than the pKa of the ionizable lipid (Hu et al., 2015; Schlich et al., 2021; Pantelić et al., 2022). Ionizable cationic lipids in LNPs are neutral at physiological pH but become protonated at pH ~6.5 in the acidic environment of the endosome (Chan et al., 2012; Suk et al., 2016). The cationic lipids then interact with the anionic endosome membrane which destabilizes the endosome membrane resulting in endosomal escape (Hafez et al., 2000; Evers et al., 2018; Buschmann et al., 2021; Schlich et al., 2021). Endosomal escape is critical to efficient nanoparticle delivery (Smith et al., 2019). LNPs that have poor endosomal escape properties remain trapped in the endo/lysosomal pathway and are consequently degraded in the acidic environment and are therefore unable to deliver their cargo (Smith et al., 2019). Mechanisms that facilitate the endosomal escape of nanoparticles include, but are not limited to, membrane fusion, osmotic pressure, nanoparticle swelling, and membrane destabilization (Smith et al., 2019).

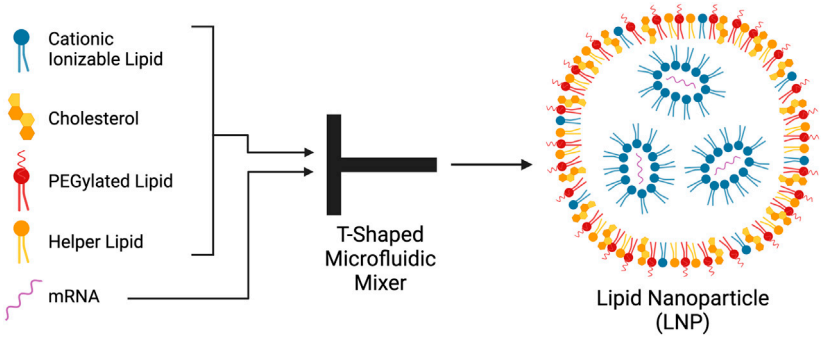

FIGURE 2
Components, structure and method of production of lipid nanoparticles (LNP). Cationic ionizable lipids cholesterol, PEGylated lipids, and helper-lipids are dissolved in ethanol and mixed with mRNA in water in a T-shaped microfluidic mixer. The lipids and mRNA self-assemble into LNPs. The negatively charged mRNA largely associates with the cationic ionizable lipids. The lipids create an outer shell surrounding the mRNA-cationic lipid complexes. Created with BioRender.com.

Helper lipids increase the stability of the nanoparticle and increase the efficiency of the delivery of the mRNA to the target cells (Cheng and Lee, 2016; Evers et al., 2018). Helper lipids often have either a conical shape or a cylindrical shape, which aids in the formation of the LNP and increases the stability of the lipid bilayer of the nanoparticle. The Pfizer and Moderna vaccines both use the helper lipid 1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC) whose cylindrical geometry aids in the formation of the lipid bilayer (Cheng and Lee, 2016). Cholesterol acts as a helper lipid, stabilizing the LNP by filling the gaps between phospholipids in the lipid bilayer, and promoting membrane fusion (Cheng and Lee, 2016; Buschmann et al., 2021). Cholesterols also function within the LNPs to improve the efficiency of endosomal escape (Cheng and Lee, 2016; Evers et al., 2018). The four components of the LNP are all crucial for its proper function when forming and when in the body. The ionizable lipids help with formation and endosomal escape, the PEG lipids shield the LNP from the immune system and increase its circulation time, the helper lipids aid in formation and stability, and cholesterol aids in membrane fusion and increases the stability of the LNP.

PEG-lipids function in the nanoparticles to shield the LNP and increase its stability in the body (Suk et al., 2016; Evers et al., 2018). PEG, a hydrophilic polymer, can be attached to molecules in a process called PEGylation. PEGylation is an established method of increasing stability and circulation of drugs in the body. They shield the LNPs from opsonins, resulting in decreased phagocytosis and preventing the LNPs from aggregating during production and in the body, resulting in longer circulation time in the body (Mui et al., 2013; Evers et al., 2018). While they increase the stability of LNPs, they can also reduce uptake by target cells and endosomal escape (Mui et al., 2013; Li et al., 2014; Evers et al., 2018). PEG lipids reduce uptake by target cells because PEG is hydrophilic, therefore reducing interactions with the hydrophobic lipid bilayer of the target cell. PEG lipids also reduce ApoE-mediated fusion required for endocytosis of the LNP into hepatocytes (Akinc et al., 2010; Mui et al., 2013). The PEGylated lipids used in the Pfizer and Moderna vaccines are 2-[(polyethylene glycol)-2000]-N,N ditetradecylacetamide and 1,2-dimyristoyl-rac-glycerol, methoxypolyethylene glycol respectively (Government of Canada). The specific reasons for why these PEGylated lipids were used is proprietary information. They are likely chosen to find a balance between stability and the ability to fuse with membranes. This balance is determined by the PEGylated lipid used and its concentration within the LNPs(Evers et al., 2018; Sarode et al., 2022).

2.2.2 Optimizing mRNA lipid nanoparticle formulations

The formulation of nanotechnologies utilized for vaccines and therapeutics determine its biocompatibility within the body. Chemical composition, degradation, metabolism, and resulting toxicological behaviours are key examples of nanomaterial properties that can influence the therapeutic efficacy, or the ability of a substance to treat the disease of concern with the most minimal side effects.

Two vaccines currently in use are the Moderna and Pfizer/BioNTech vaccines (29); both showing efficacies of around 95% (Schoenmaker et al., 2021). Despite these high efficacies, analysis of antibody and T-cell responses of 122 individuals 6 months after two doses of the Pfizer vaccine revealed the median levels of S-RBG IgG dropped to 7% of their peak potencies (Naaber et al., 2021). This is indicative of short-lived immunity prompting the need for more subsequent booster doses. Aside from this, a wide variety of side effects on the range of less severe to adverse have been experienced by individuals who have received these mRNA-LNP vaccines.

Common side effects of nanomaterial-based vaccines, particularly those that utilize mRNA-LNP formulations include swelling at the location of injection, fatigue, fever, and headache (Ciotti et al., 2020; Morens et al., 2020; Anand and Stahel, 2021) A study conducted by Ndeupen et al. (2021), where empty lipid nanoparticles formulated with phosphate buffered saline was injected in mice, found intense inflammation caused by the lipids which could possibly lead to side-effects in mRNA vaccines. More adverse side effects include hypersensitivity reactions that are commonly attributed to the PEGylated (polyethylene-glycol) lipid component shown to stimulate larger complement activation (Senti et al., 2022) pre-existing antibody responses (Ju et al., 2022), and activation of allergy specific immune cells (Troelnikov et al., 2021). Although the addition of PEG has been known to play a beneficial role in nanoparticle stability and improving specificity for uptake of targeted cell types (Bigini et al., 2021), further studies have yet to thoroughly quantify the extent to which it contributes to stimulating these adverse immune responses. Experiments conducted by Tanaka et al., 2021 demonstrate how lowering the PEG-lipid content from 3% to 0.75% alters the surface density of LNPs and results in enhanced transfection of mRNA through the biological membrane (Tanaka et al., 2021). Hence, application of these PEG-related molecular modifications can be a way to optimize current LNP formulations in the SARS-CoV-2 vaccines.

To optimize the formulation of the LNPs used in COVID-19 vaccines, thousands of preliminary lipid formulations have been tested in search of sufficient lipid ratios for the mRNA carriers (Pantelić et al., 2022). There are many factors which affect the properties of the LNPs that change based on which components and ratios of them are used. Properties that frequently change among different LNP formulations are individual size and size variability within a batch (batch-to-batch variability) (Evers et al., 2018; Maeki et al., 2022). It has been shown that the size of the LNPs used in an mRNA vaccine can impact the immunogenicity of the vaccine (Yan et al., 2013; Hassett et al., 2021; Okuda et al., 2022). LNPs typically range from ~30 to 200 nm, and within this range, the immunogenicity of the LNPs changes. For example, a study conducted by Hassett et al., in 2021 found that nanoparticles around 100 nm in size were able to recruit sufficient amounts of immune cells for participation in antigen presenting cell (APC) uptake for mRNA delivery. This study indicates that factors like immune cell recruitment and interactions with APCs can be influenced by particle size which consequently affect the degree of antigen expression as a result of mRNA-LNP delivery to host cells (Hassett et al., 2021).

Adjustment of other molecular components within LNPs can also serve to address challenges with nanoparticle stability and long-term effects of mRNA-based vaccines. In fact, a study conducted by Xu et al., 2022 confirms how the geometry and molecular chirality properties of nanoparticles conferred by its composition greatly influences the cellular uptake and lysosomal escape of nanostructures by the immune system (Xu et al., 2022) and, therefore, its therapeutic efficacy. Similarly, Alvarez-Benedicto et al., 2022 highlights the chemical properties like phospholipid head group charge, or the type of lipid DSPC or DOPE, within LNPs that mediate the destination of mRNA payloads either to the spleen or liver; they were also able to observe which phospholipid component in LNPs are more likely to contribute to endosomal escape after endocytosis (Alvarez-Benedicto et al., 2022). In terms of mRNA compatibility and stability with LNPs, a study by Packer et al., 2021 found that the presence of mRNA impurities caused by ionized lipids could disrupt mRNA translation and therefore, can negatively impact the efficacy of mRNA-lipid-based products (Packer et al., 2021). Experiments conducted by Cornebise et al., 2022 further illustrate the significant influence of lipid components of LNPs on their mRNA payload through the discovery of amino lipids that form stabilizing hydrogen bonds with the mRNA resulting in enhanced *in vivo* delivery (Cornebise et al., 2022). (Cornebise et al., 2022)

Because of the unstable nature of current COVID-19 vaccines and their requirement to be stored at low temperatures (Schoenmaker et al., 2021), there has been recent research into preserving the stability of the mRNA-LNPs without altering its chemical composition. One technique for LNP preservation, as employed by researchers Muramatsu et al., 2022, is freeze-drying or lyophilization where structural integrity from particle diameter, zeta potential, mRNA loading concentration, to encapsulation efficiency under several storage conditions was maintained without affecting the mRNA translation *in vivo* (Muramatsu et al., 2022). Despite LNPs ability to maintainin their structural integrity and to encapsulate a broad range of payloads, current efforts to optimize mRNA-LNP vaccines to address variants of concern are primarily achieved through modulating mRNA sequences as these mRNA constructs can easily be administered for critical evaluation.

2.3 Lipid nanoparticle production and manufacturing techniques

LNPs are most commonly produced using rapid mixing (Evers et al., 2018; Maeki et al., 2022) In this method, the lipid particles are dissolved in an organic solvent solution, such as ethanol, and the mRNA is dissolved in an aqueous buffer solution. These solutions are rapidly mixed and the LNPs self-assemble. At commercial scales, T-shaped microfluidic devices are used in the production of the currently approved vaccines for COVID-19 as they allow for large batches to be made (Cheng and Lee, 2016; Pantelić et al., 2022) In a T-shaped microfluidic device, the ethanol and buffer mix together at the junction of the T shaped channel (Maeki et al., 2022), as shown in Figure 2. Here, the LNPs self-assemble

from the lipids and the mRNA which are collected at the end of the exit channel. As previously mentioned, this takes place at an acidic pH below the pKa of the ionizable cationic lipid. The cationic ionizable lipids form electrostatic interactions with the negatively charged mRNA which aids in encapsulation efficiency. The components also form a shell around the inner ionizable lipid membrane enclosing the mRNA (Albertsen et al., 2022; Maeki et al., 2022; Pantelić et al., 2022).

LNPs produced for these mRNA vaccines are of a consistent size as it affects several aspects of cargo delivery to the targeted cells as previously mentioned. It has been found that fast mixing of the aqueaus and ethanol solutions is associated with the production of smaller nanoparticles and slow mixing is associated with larger LNPs (Maeki et al., 2022). Mixing of the ethanol and aqueous solutions so that there is a consistent ratio of both phases' results in little variability between the sizes of LNPs while a ratio gradient refers to size variability outcomes. Microfluidic devices are tuned to have little variability in the size of LNPs and are also tuned to create the desired size of nanoparticles. Previously used dilution methods such as vortexing have large variability in the size of the LNPs produced. This is because the mixing is very] non uniform in this method resulting in variously sized LNPs. Research is being done on improving the process of making nanoparticles. One such company is Precision Nanosystems (Vancouver, B.C.). Their NanoAssemblr® technology decreases the variability in size of nanoparticles compared to a typical T shaped microfluidic device as demonstrated in a study that encapsulated silencing mRNA (siRNA) in LNPs for therapeutic purposes (Walsh et al., 2014).

2.4 Current available vaccines to address emerging variants of SARS-CoV-2

Emerging variants of SARS-CoV-2 demand the production of effective vaccines to protect against them. SARS-CoV-2 variants have been associated with increased disease severity, transmissibility, and the ability to avoid detection by the adaptive immune system (Tao et al., 2021). Mutations occurring primarily in the spike protein of SARS-CoV-2 have resulted in new VOCs of the virus.

Variants of SARS-CoV-2 were first discussed as early as April 2020 (Forster et al., 2020) and these variants were first detected in late 2020 (Tao et al., 2021). Notable VOCs include the Alpha, Beta, Gamma, Delta, and Omicron variants, which differ from each other and the originalstrain of SARS-CoV-2 mainly by various mutations in the spike glycoprotein (Hadj Hassine, 2022b). A high incidence of point mutations in the N-terminal domain (NTD) and receptor binding domain (RBD) of the spike gene suggests selection for increased viral fitness *via* changes in the spike protein that improve host-receptor binding or the ability to evade host antibodies (McLean et al., 2022). Other viral characteristics affected by spike glycoprotein mutations include infectivity, transmissibility, pathogenicity, antigenicity, as well as immune escape shown in Figure 3 (Harvey et al., 2021). In 2020 and 2021, the Alpha, Beta, and Gamma VOCs drove worldwide surges of COVID-19 infection until the rise of the Delta variant, which displaced all other VOCs as the dominant variant (Tao et al., 2021; Viana et al., 2022). The Omicron variant of SARS-CoV-2 was first detected in November 2021 and within 3 weeks spread to 87 countries (Viana et al., 2022). Omicron, characterized by its ability to rapidly spread between vaccinated individuals, quickly proceeded to replace the Delta variant as the dominant variant (Tao et al., 2021; Viana et al., 2022). Currently these variants are being addressed through acquiring booster doses, and modifications to vaccine formulation specific to mRNA cargo, alongside enhancement of adjuvant properties through the addition of nanomaterials such as liposomes to non-mRNA vaccine types.

2.4.1 Efficacy of booster doses against SARS-CoV-2 variants

The two nanoparticle-based vaccines in use in Canada are effective against COVID-19 variants (Liu J. et al., 2022). One study showed that two doses of Pfizer's BNT162b2 vaccine elicited the neutralization of Delta plus, Lambda, Mu, B.1.1.519, and Theta SARS-CoV-2 variants (Liu J. et al., 2022). Subsequent doses of mRNA COVID-19 vaccines, coined booster shots, have been recommended to restore wanning immunity over time and as protective tools against the loss of vaccine efficacy from emerging COVID-19 variants (Scheaffer et al., 2022). Emphasizing the importance of booster shots, studies have shown that three doses of Pfizer's BNT162b2 vaccine and Moderna's mRNA-1273 SARS-CoV-2 vaccine elicit improved neutralizing antibody activity and protection against the SARS-CoV-2 Omicron variant when compared to two doses (Andrews et al., 2022; Ariën et al., 2022). Variant-specific mRNA booster vaccines are also in trial, showing promising results in mouse models (Flemming, 2022). For example, one study demonstrated that immunizing mice with a booster vaccine of an Omicron-specific version of Moderna's mRNA vaccine elicited improved protection against the Omicron variant than being boosted by the original version of the mRNA-1273 COVID-19 vaccine, demonstrating the enormous potential of altering mRNA sequences in mRNA vaccines to address COVID-19 variants (Flemming, 2022; Gagne et al., 2022). In early September 2022, Health Canada approved for adults the Moderna Spikevax Bivalent COVID-19 booster, an mRNA vaccine that includes the mRNA sequence from the original COVID-19 strain and the Omicron BA.1 variant (doi:10.1136/bmj.o2144). This booster is predicted to offer protection against further emerging subvariants of the Omicron variant (Dyer, 2022).

Booster doses may not exhibit consistent efficacy in populations Older adults may have a weaker immune

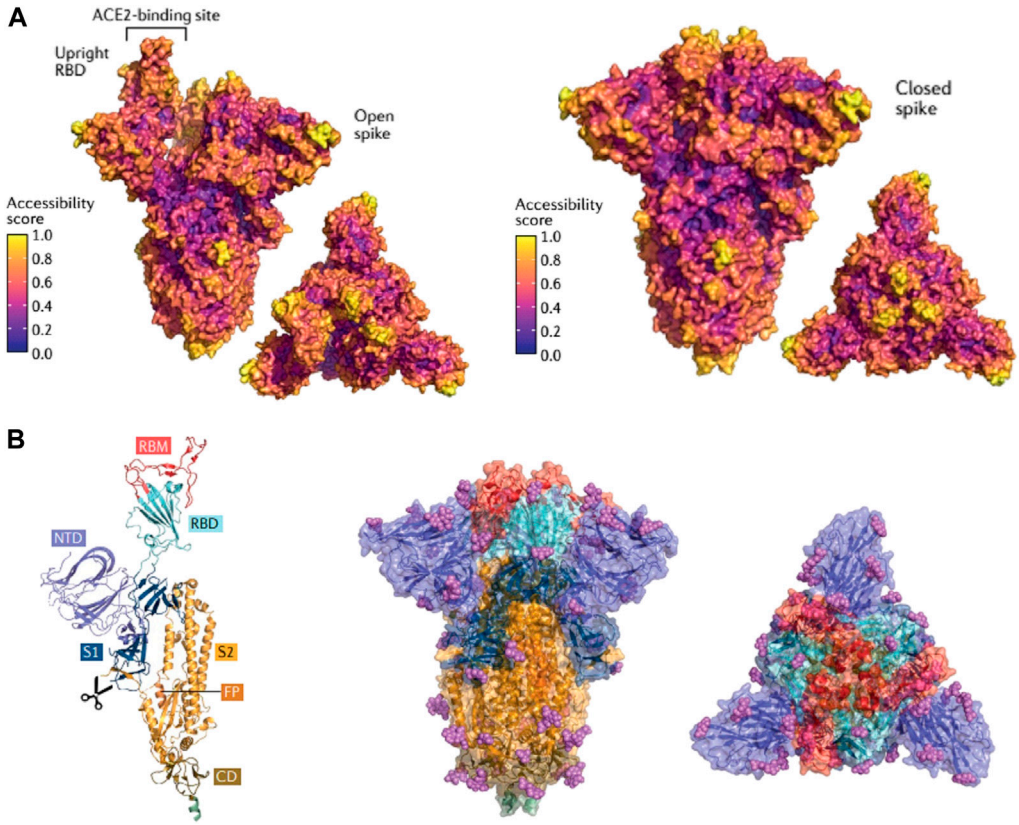

FIGURE 3
SARS-CoV-2 Spike protein structures illustrating regions specific for antibody binding and domains prone to specific amino acid mutations responsible for emerging variants of concern synthesized by researchers (Harvey et al., 2021). **(A)**. SARS-CoV-2 Spike protein in an open conformation and closed conformation exhibiting key areas of antibody binding based on conformational epitopes. Colors are based on antibody accessibility scores where high values on the scale indicate higher antibody recognition. Lower values of antibody accessibility indicate a lower antibody recognition which strongly correlates to domains containing amino acid mutations characteristic of emerging variants of concern. **(B)**. SARS-CoV-2 Spike Protein composition: RBM (receptor binding motif), RBD (receptor binding domain), NTD (amino-terminal domain), S1 (subunit 1, part of NTD and RBD responsible for binding ACE2), S2 (Subunit 2, part of trimeric protein core responsible for membrane fusion). This figure is reprinted with permission from Nature Springer from (Harvey et al., 2021).

response and faster declining antibodies than younger adults; recent data found that adults 80 years and older have lower antibody elicitation and titres than younger adults under the age of 60 years old when vaccinated with two doses of the Pfizer/BioNTech Comirnaty COVID-19 vaccine (Muller et al., 2021). Due to this low efficacy, older adults may have a need for a greater number of booster doses to maintain protection against severe disease as a single booster dose may not provide sufficient protection as it would in a younger adult. Distributing booster doses to communities with a high proportion of elderly individuals or immuno-deficient individuals has been a strategy used to maintain adequate protection in vulnerable populations (Galanis et al., 2022).

Further, in communities, concern of vaccine safety and side effects are critical factors in booster dose refusal and henceforth, distribution (Galanis et al., 2022). Recent analysis of voluntary reports to the Vaccine Adverse Events Reporting System (VAERS) in the United States between September 2021 and February 2022 revealed 39,286 cases of adverse reactions to a booster dose of an mRNA COVID-19 vaccine and revealed that adverse reactions are more frequent when receiving a heterologous booster (e.g., Moderna Spikevax for the first two doses and Pfizer/BioNTech Comirnaty the booster) than a homologous one (e.g. Moderna Spikevax for the first two doses and the booster dose) (Hause et al., 2022) It is possible that hesitant individuals may be more willing to receive vaccines if they were given the choice of homologous boosting. Adequate education about age group-dependent efficacy and booster side effects should remain in practise during booster dose distribution.

2.4.2 Optimizing vaccine formulations *via* mRNA modifications against SARS-CoV-2 variants

According to a 2021 review, Pfizer/BioNTech and Moderna's mRNA-LNP vaccines have demonstrated a

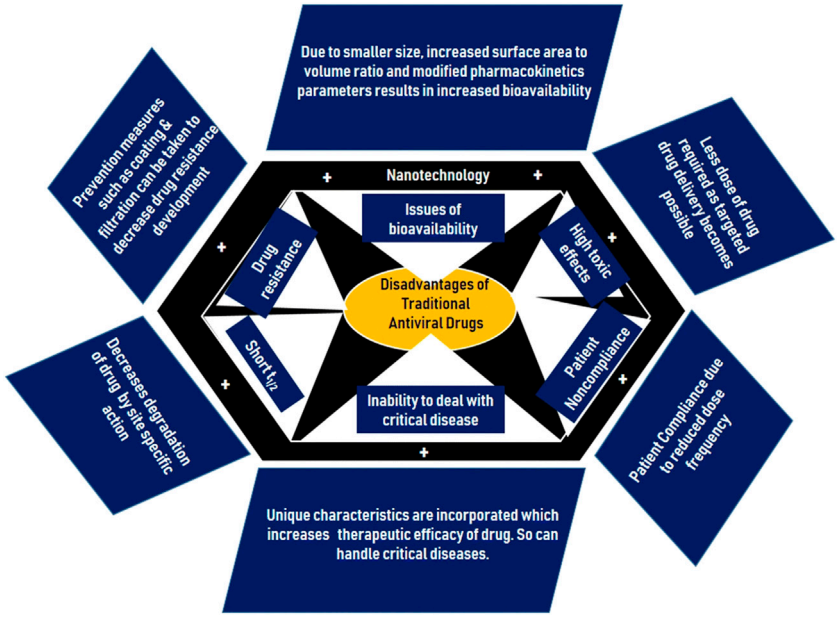

FIGURE 4
Summary of the shortfalls of traditional antivirals and methods of revision with nanoparticles (Chakravarty and Vora, 2021). Permission to use this figure is available. This figure is reprinted with permission from Springer Nature from (Chakravarty and Vora, 2021).

greater than 90% retention of efficacy in preventing disease towards the original D614G and alpha strain of SARS-CoV-2 but has shown a lower efficacy of approximately 80% in preventing disease alongside infection for other variants like Beta, Gamma, or Delta (Kandikattu et al., 2021). This is indicative of how mRNA-LNP vaccines induce broad based immunity. A 2022 systematic review and meta-analysis of COVID-19 vaccine effectiveness against SARS-CoV-2 variants of concern also details the same trends from clinical trial data and ongoing vaccination programs showing a consistent decrease in vaccine effectiveness for LNP based vaccines (Zeng et al., 2022).

To address the mutated RBD and NTD regions in the SARS-CoV-2 spike protein in variant strains, the robust properties of LNP based formulations are utilized to deliver specific modified mRNA sequences. The nature of the mRNA transcript used in the vaccine allows its sequence modification so that an altered mRNA transcript, specific to a COVID-19 variant, can be loaded in an LNP for delivery, which is extraordinarily advantageous for tackling COVID-19 variants (Forchette et al., 2021). For example, designed mRNA encoding for specific RBD proteins rather than the full-length S protein of SARS-CoV-2 and assessed the vaccine efficacy through cellular immune responses in mice for wild-type SARS-CoV-2 along with variants Delta and Omicron. As a result, Liu et al. (2022) experiments illustrated strong neutralizing antibody responses and significant protection from two to three doses against wild-type, Delta, and Omicron variants (Liu C. et al., 2022). This study further serves as an example to how mRNA modifications could increase specificity in immune responses through presentation of an immunogen rather than the whole antigen. Variant-specific vaccination using encapsulated mRNA in LNPs designed to include mutations in the spike protein have also been investigated (Peng et al., 2022; Zhang et al., 2022), although the resulting levels of neutralization vary, which poses a challenge when formulating a succinct, universal, one-dose vaccine against COVID-19.

2.4.3 Nanomaterial additions to non-mRNA vaccine types

mRNA-LNP vaccines offer several advantages over other vaccine types in the light of emerging COVID-19 variants by being cost-effective, and rapidly synthesized. Many LNPs are self-adjuvanting due to the nanomaterials they are composed of andexhibit adjuvanting activity, which is the ability to enhance an immune response towards an antigen (Alameh et al., 2021; Igyártó et al., 2021). An adjuvant is any ingredient that creates a stronger immune response to a vaccine. The cholesterol and phospholipid used in LNPs also occur in mammalian cell membranes, making the possibility of triggering inflammation or an innate immune response unlikely (Igyártó et al., 2021). The adjuvating properties of LNPs thus likely stem from other nanomaterial components of the LNP, such as the inflammatory properties of the cationic ionizable lipid, the mRNA, or both (Alameh et al., 2021). In mice, a LNP formulation used to deliver mRNA for immunization against COVID-19 was shown to exhibit intrinsic adjuvant activity,

leading to the promotion of T follicular helper cell, germinal center B cell, long-lived plasma cells, and memory B cell responses for antibody production (Alameh et al., 2021).

Addition of nanomaterials commonly comprising LNPs can increase adjuvant properties to non-mRNA vaccine types. For example, liposome adjuvants are a cost-effective and rapidly made solution (Abhyankar et al., 2021) that may contain nanomaterials, phospholipids or cholesterol components, similar to LNPs that enhance other vaccine types like protein subunit vaccines specific for COVID-19 variants. In a mouse model of COVID-19, a spike protein vaccine containing a dual Toll-like receptor ligand nanoliposome adjuvant was shown to elicit anti-spike neutralizing antibody responses and protect mice from lethal COVID-19 infection and lung immunopathology (Abhyankar et al., 2021). Similarly, NARUVAX-C19, a recombinant spike protein vaccine candidate formulated with a nanoemulsion oil adjuvant, was shown to produce high levels of neutralizing antibodies against wildtype and delta COVID-19 variants (Tabynov et al., 2022).

Liposomes similar to LNPs can also be utilized to deliver the SARS-CoV-2 spike protein antigen as part of the nanoparticle formulation. In a separate mouse model of COVID-19, nanoparticles structurally comprised of liposomes that were decorated with either SARS-CoV-2 spike protein or its receptor binding domain on its surface exhibited effective adjuvant properties and high thermal stability (Mabrouk et al., 2021). The material formulation of the vaccine also included cobalt porphyrin-phospholipid for particle induction and the adjuvants QS-21 and PHAD, a synthetic monophosphoryl lipid A. The high thermal stability demonstrated in this paper was due to the lyophilisation or freeze-drying of the particle to preserve the nanoparticle-liposome structure alongside the additional adjuvant properties of the liposome to provide stability *in vivo* upon administration. The nanoparticle vaccine produced by this group exemplifies how the incorporation of additional nanomaterials or components like liposomes in vaccine formulation can make vaccines easier to store than current Pfizer and Moderna vaccines as thermostable vaccines are ideal for global distribution as well as storage in areas experiencing high case volumes of COVID-19 variants.

3 Nanotechnology advancements in developing therapeutics

3.1 Defining the need for SARS-CoV-2 therapeutics

Due to the implausibility of fool-proof prophylactic measures, health care professionals sought treatments to combat COVID-19 post infection. Where vaccines provide acquired immunity to transmissible pathogens, therapeutics can prevent viral replication and work to bolster the body's immune response following infection (Rehman et al., 2020). In the early pandemic, doctors pivoted towards broad-spectrum antivirals with well-studied safety profiles to test for therapeutic potential against SARS-CoV-2 (Weiss et al., 2020a). However, antiviral treatments present new challenges, such as adverse side effects from interactions with prescription medications, low bioavailability, and selectivity as highlighted by Figure 4 (Chakravarty and Vora, 2021). Thus, treatments adopted nanotechnological strategies to develop safer drug-delivery systems and improve the therapeutic index (TI) of existing drug candidates (Zhou et al., 2021).

Nanotechnology in therapeutics is not a novel concept, however previous applications focused mainly on cancer treatments, specifically to overcome the dose-limiting toxicity of many chemotherapy agents (Sinha et al., 2006). The use of nanoparticles in tandem with currently available medications minimizes potential drug toxicity or deleterious effects through targeted drug delivery systems that ultimately increase drug bioavailability at specific sites or tissues. Furthermore, administering medications *via* nanoparticles does not require dilution of the compounds. Previous problems arose when following dilution: medications lost their effectiveness due to virus-compound dissociation, allowing the virus to regenerate. However, nanoparticles have the potential to inflict permanent damage to the virus (Weiss et al., 2020a; Cavalcanti and Nogueira, 2020).

Pivoting to the overall benefits of nanoparticle-based COVID-19 therapeutics, the biggest draw of such treatments is their ability to effectively deliver medications while limiting the risk of side effects by serving as drug carriers. There are two major strategies to achieve this, the first being their ability to lower the overall dose of a drug through refining the region of administration (i.e., targeting more specific regions of infected tissues). Such a tactic reduces the chance of harmful drug agents aimed at diseased cells from contacting healthy ones (Xiao et al., 2022). The second method focuses specifically on the delivery of hydrophobic medications, which often require harmful solubilizing agents. One study looking at celastrol, a compound demonstrating anticancer activity, found a way to bypass the use of toxic solubilizing agents using celastrol liposomes. The liposomal envelopment of the hydrophobic drug solved the problem of low water solubility by embedding within the lipid bilayer, while hydrophilic drugs can exist within the aqueous core of the particle (Wolfram et al., 2014) Such success could by expanded for COVID-19 therapeutics.

Limited research exists detailing the efficacy of nanotechnological therapies against emerging COVID-19 variants of concern such as Omicron, Delta, and BA.5. Antivirals such as Remdesivir (G7S-5734) are a prominent treatment choice following inhibition of SARS-CoV-2 and MERS-CoV replication in various *in-vitro* environments

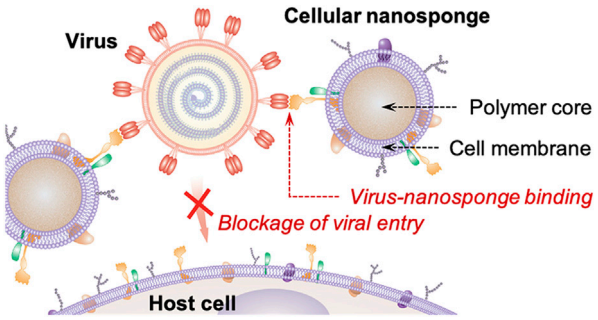

FIGURE 5
Depiction of nanosponges mimicking the target cell (host cell) membranes consisting of similar receptors (CD147 and ACE2). This inhibits the interaction of the virus with the host cell resulting in the blockage of viral entry into the host cell. This image is adapted from (Zhang et al., 2020) under the ACS Author's Choice usage agreement. This is an unofficial adaptation of an image that appeared in an ACS publication. ACS has not endorsed the content of this adaptation or the context of its use.

(Sheahan et al., 2017). Similarly, chloroquine and hydroxychloroquine, two antimalarials that rose exponentially in popularity at the start of the pandemic, remain an option due to their ability to prevent pneumonia exacerbation in acute COVID-19 infections, though cardiotoxicity associated with the compounds is a concern (Alhajj and Gencer, 2020). Though chloroquine was not produced specifically to treat SARS-CoV-2, a standard dose of the drug impedes viral replication (limiting COVID-19 entry into cells) and supports the immune system through activating cellular anti-inflammatory responses.

In contrast to the previously stated antivirals, Paxlovid has been a standout COVID-19 therapy for treating mild to moderate cases since gaining Emergency Use Authorization (EUA) by the United States Food and Drug Administration (USFDA) in December 2021 (Najjar-Debbiny et al., 2022). Produced by Pfizer, the drug is a combination of nirmatrelvir (a SARS-CoV-2 protease inhibitor) and ritonavir (an HIV protease inhibitor that impedes cytochrome P450 and P-glycoprotein, thus increasing the half-life of nirmatrelvir) (Islam et al., 2022; Najjar-Debbiny et al., 2022). Researchers found that when given Paxlovid within the first 5 days of SARS-CoV-2 infection, progression to acute COVID-19 and mortality decreases significantly. More importantly, the findings from Israel during the height of Omicron wave demonstrated Paxlovid has potential to treat COVID-19 subvariants (Najjar-Debbiny et al., 2022). Though Paxlovid itself is not considered a nanotechnology, it is worth noting as it was produced specifically for COVID-19 and could hold further potential as a broad-spectrum antiviral. Furthermore, it has a thorough safety profile and is easier to produce than more complex nanotechnologies, allowing for a long-term and cost-effective method to managing COVID-19 in everyday life (Wen et al., 2022). It could also be combined with other nanotechnologies to provide controlled release to treat COVID-19, reducing dose frequency and limiting the potential for over usage.

Though nanotechnology improves the efficacy (and mitigates adverse side effects) of antiviral treatments, further research and funding is needed to make them a viable option for those suffering from COVID-19. Presently available therapeutic nanomedicines are not specific to SARS-CoV-2, with most research drawing on their success in cancer, Ebola, or HIV treatments. Furthermore, a cost-effective production strategy has yet to be proposed, especially one on a large enough scale for a worldwide population (as most studies using such technologies are pre-clinical and do not require an abundance of materials) (Alhajj and Gencer, 2020).

3.2 Mechanisms of action employed by SARS-CoV-2 specific therapeutics

Studies focusing on employing nanotechnology to develop COVID-19 therapeutics have found some promising evidence supporting their role in eradicating SARS-CoV-2. One of the most common strategies implemented for SARS-CoV-2 therapeutics is blocking the interaction between the viral spike protein and ACE-2 receptor. Furthermore, nanosponges and nanodecoys have been shown to effectively block the viral entry into the cells by blocking this interaction (Caldera-Crespo et al., 2022). Similarly, researchers have also employed ways to neutralize viral proteins and enzymes along with dampening the host cytokine responses to prevent tissue damage. Utilizing nanobodies, gene delivery systems involving nanocapsules, metal nanoparticles, inorganic and organic nanoparticles, and quantum dots are some of the interesting applications of nanoparticles in neutralizing viral enzymes and reducing cytokine storms in the host to reduce inflammation. Although

these therapeutic approaches would have some side effects, their potential in combating SARS-CoV-2 cannot be neglected.

3.2.1 Blocking ACE2 host cell receptor binding

Therapeutic approaches against COVID-19 requires blocking the interaction between the viral spike protein with the angiotensin-converting enzyme (ACE2) receptor of the cells to prevent the virus from entering host cells (Weiss et al., 2020b). Zhang *et al.* developed novel cellular nanosponges by coating human cell-derived membranes onto poly (lactic-*co*-glycolic acid) (PLGA) nanoparticle cores to divert SARS-CoV-2 from entering their usual cell targets as illustrated in Figure 5 (Zhang et al., 2020). Nanosponges express similar receptors to the target cells allowing the virus to interact with CD147 and ACE2 receptors to enter the cells. The ACE2 receptor is highly expressed in Vero E6 cells isolated from the kidney cells of African green monkey making this cell line ideal for use as an infection model for COVID-19. The expression of host serine protease TMPRSS2 is then induced in this cell line as it is responsible in cleaving viral glycoprotein to activate the virus (Mollica et al., 2020). Experiments conducted in this *in vitro* study demonstrated that the entry of live SARS-CoV-2 virus into Vero E6 cells was neutralized by human lung epithelial type II cell nanosponges and human macrophage nanosponges in a concentration dependent manner. Similarly, PLGA nanoparticles have been investigated for their role in drug delivery to administer therapeutics against SARS-CoV-2. An *in vitro* kinetic study done by Burcu *et al.* focused on controlled release of Oseltamivir phosphate (OP), an antiviral model drug, when loaded onto PLGA NPs conjugated to spike-binding peptide 1(SBP1) of SARS-CoV-2 (Ucar et al., 2021). The goal was to prevent the entry of the virus by using SBP1 conjugated NPs as SBP1 peptide interacts with the receptor binding domain (RBD) of SARS-CoV-2. Thus, this approach of controlled drug release prevents COVID-19 infection. The study reported steady release rates of 53.7% and 50.4% after 72 days for OP loaded NPs and OP loaded NPs conjugated to SB1 indicating the potential of this novel controlled drug delivery system to tackle COVID-19.

3.2.2 Mediation of inflammation through gene delivery using nanomaterials

The need for COVID-19 therapeutics has led to numerous studies exploring the potential of nanovesicles, nanocapsules, and nanoparticles as drug delivery systems. Immune response induced by SARS-CoV-2 leads to the production of reactive oxygen species (ROS), cytokines, and proteases in COVID-19 patients leading to hyperinflammation (Qin et al., 2020). It is imperative to treat the overproduction of ROS as it could otherwise lead to an increased viral replication advancing the cells to apoptotic stage of COVID-19 infection. Qin *et al.* designed nanocapsules made of 2-methacryloyloxyethyl phosphorylcholine (MPC), N-(3-aminopropyl) methacrylamide hydrochloride (APM), and N,N′-methylenebisacrylamide (BIS) monomers (called n (CAT)) that encapsulates catalase enzyme to ubiquitylate ROS (H_2O_2). Utilizing nanocapsules to encapsulate the enzyme led to a 16.8-fold increase in the half-life of the encapsulated enzyme *in vivo*. The results indicate 100% cell viability when $1{,}000 \times 10^6$ M H_2O_2 was added to human pulmonary alveolar epithelial cells (HPAEpiC) with n (CAT) when compared to 63% viability of control cells. Notably, eliminating H_2O_2 dampened the downstream ROS production *in vivo*. This led to the repression of SARS-CoV-2 replication in rhesus macaques further elucidating n (CAT)'s role in protecting tissues from oxidative injury, reinvigorating injured cells, and immunoregulation.

3.2.3 Limiting the binding of viral particles using nanodecoys and nanobodies

Another strategy employed to target COVID-19 uses decoy nanoparticles or nanodecoys that are designed to mimic cellular targets to inhibit virions. Taking advantage of ACE2 expressing lung spheroid cells (LSC), Zhenhua *et al.* designed LSC-membrane nanovesicles to neutralize intranasally delivered SARS-CoV-2 in cynomolgus macaques (Li et al., 2021). The study reported that the presence of sub genomic RNA (sgRNA) in bronchoalveolar lavage decreased from 6.243 $\log_{10}$ RNA copies ml^{-1} in control animals to <1.70 $\log_{10}$ in nanodecoy treatment groups. Similarly, immunohistochemistry (IHC) data proved a decrease in SARS nucleocapsid protein (SARS-N) in LSC-nanodecoy treatment groups in lung tissues along with a reduction in viral replication as evident from the RNAscope data, suggesting the potential of this technology in reducing the effects of COVID-19.

New COVID-19 VOCs provide challenges when designing therapeutics employing decoy strategies. Gunnels *et al.* identified a Beta mutant consisting of three mutations in its receptor binding domain making it resistant to FDA approved mAbs (K417N, E484K, N501Y) that inhibit its interaction with the host ACE2 (Gunnels et al., 2022). These mutations increase affinity of the spike protein for the host ACE2 by three folds. Another variant tested in the study was F486S that contains point mutation in the spike receptor binding domain decreasing its affinity for ACE2 receptor. They utilized dose response curves to analyze if the decoy extracellular vesicles containing ACE2 (obtained *via* ultracentrifugation (UC)) would inhibit Spike-lenti variants. The results indicated inhibition of Spike-lenti variants (Beta and F486S) *via* decoy UC-EVs (including design variations) that have evolved to be resistant against mAbs. These results remained the same when UC-EVs were used to inhibit Delta (L452R, T478K), Delta-plus (K417N, L452R, T478K), and Lambda (L452Q, F490S) variants.

A recent Cryo-EM study has discovered an epitope on the spike protein that is conserved in major variants of SARS-

CoV-2 along with an antibody fragment VH Ab6 that can neutralize the variants by interacting with the epitope (Mannar et al., 2022). Though more sensitive to some than others, VH Ab6 confers tolerance to variants including Alpha, Beta, Gamma, Delta, Kappa, Epilson, and Omicron due to its biochemical properties, making this fragment tolerant to RBD mutations. This study opens doors for soluble mAb therapies amongst others. Interestingly, it could potentially be combined with the nanodecoy strategy mentioned above to generate VH Ab6 coated nanoparticles to neutralize SARS-CoV-2. Even though evidence does not currently exist for this particular antibody-nanodecoy strategy, there are numerous studies on developing antibody conjugated nanoparticles to treat various diseases (Juan et al., 2020). Another strategy that has become popular recently is utilizing nanobodies to neutralize the spike protein of SARS-CoV-2. Nanobodies are obtained from camelids and sharks and only consist of one variable heavy chain domain (VHH) (Mahmud et al., 2022). In contrast to human IgGs, their small size of 12–15 kDa not only allows greater permeability, but also allows for a greater interaction efficiency within the protein grooves. A recent study by Fu *et al.* constructed humanized nanobody library and one of the nanobodies RBD-1-2G neutralized a psuedotyped particle mimicking N501Y mutation as seen in the receptor binding domain of SARS-CoV-2 variant B.1.1.7 (Fu et al., 2022). The study utilized these nanobodies to specifically target the spike-ACE2 interactions and hence, preventviral entry.

3.2.4 Delivering siRNA anti-viral therapies using stealth lipid nanoparticles

Research studies have further emphasized the role of nanoparticles as carriers of gene delivery therapies to treat COVID-19. Gene delivery therapeutics ultimately focus on ceasing disease progression. Some of the nucleic-acid based therapeutic strategies include silencing the viral proteins post transcriptionally using RNA interfering therapies, inhibiting protein function *via* aptamers, and inhibiting viral replication using DNAzymes (Piyush et al., 2020). Since the genome of SARS-CoV-2 is encoded by RNA, short double stranded RNA molecules called small interfering RNA (siRNA) can be utilized to knock out the virus by silencing the genes transcriptionally or post transcriptionally (Idris et al., 2021). Idris *et al.* have developed a stealth lipid nanoparticle (sLNP) delivery system to screen three siRNA candidates (siUTR3, siUC7, and siHel1) that target conserved regions of SARS-CoV-2 *in vivo*. Their novel formulation involved a reduced use of cationic lipid particles DOTAP (~40%) to reduce toxicity and incorporating ionizable lipid MC3 which helps in the release of siRNA. The DiD labelled DOTAP/MC3 LNP-siRNAs (~80 nm) were found to localise in lungs, liver and spleen and outgrowth analysis from lungs after 7–8 days post infection suggested repression of SARS-CoV-2 *in vivo*. Such novel methods re-emphasize the immense potential of nanoparticles in COVID-19 therapeutics.

3.2.5 Neutralizing viral components

3.2.5.1 Using inorganic nanoparticles

Investigations exploring the role of metal and theranostic nanoparticles, and quantum dots have reported promising findings that bring nanoparticles one step closer to clinical trials for COVID-19 treatment. Gold nanoparticles are of interest due to their biocompatibility and poor immunogenicity. Labouta et al. (2021) proposed a novel plasmonic photothermal therapy (PPT) based on theoretical discrete dipole approximation that utilizes ACE-2 functionalized gold nanorods (AuNRs) to target the virus which is followed by irradiation with near-infrared (NIR) light *via* flexible bronchoscopy. The study provides mathematical evidence to prove that the tip-to-tip configuration of AuNR absorbs NIR leading to the formation of strong field hotspots leading to localized heat generation to deform the virus bound to AuNRs. In addition, Auranofin is a drug that was approved for rheumatoid arthritis and it is a gold-containing triethyl phosphine (Iraci et al., 2022). A study conducted using human Huh7 cells to investigate its inhibitory impact on SARS-CoV-2 demonstrated inhibition at very low concentrations along with a reduction in cytokine production. Auranofin induces apoptosis by inhibiting essential redox enzymes leading to oxidative stress (Gil-Moles et al., 2020). Copper nanoparticles are of interest to researchers to combat SARS-CoV-2 due to its antiviral properties. Cu^{2+} ions are effective in producing ROS which are harmful to the viral genome as they deactivate viral enzymes. Almaki *et al.* utilized Co. (II) and Cu (II) complexes to produce a thiazole derivative that interacts with 6lu7 and 7bz5 proteins of SARS-CoV-2 that could be used as an antiviral treatment (Almalki et al., 2021).

Another molecular docking study on iron oxide nanoparticles (FDA approved for the treatment of anemia) found that Fe_2O_4 formed a stable complex with S1 subunit of receptor binding domain of the SARS-CoV-2 due to the formation of 4 hydrogen bonds (binding free energy of −10.66 kcal/mol) (Abo-zeid et al., 2020). The higher affinity of SARS-CoV-2 for IONPs inhibits its interaction with ACE2 host receptors emphasizing the potential of IONPs as antiviral therapeutic agents against this virus. Figure 6 taken from (Chenthamara et al., 2019) provides a broader perspective on the different roles of iron oxide nanoparticles for biomedical applications. Potential toxic effects of IONPs have been investigated and further research needs to be performed before its consideration as an effective therapeutic against viral pathogens like SARS-CoV-2 and as drug delivery agents (Martins et al., 2021). In 2011, an experiment was performed where the toxic effects of IONPs were tested for both *in vivo* (Wistar rats) and *in vitro* environments (Szalay et al., 2012). The

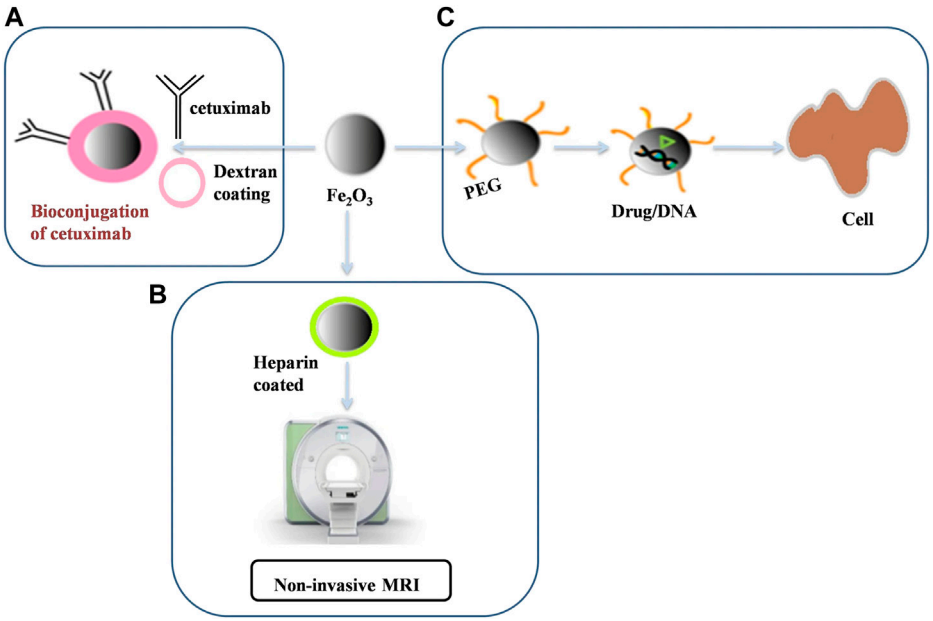

FIGURE 6
Summary of the use of iron oxide nanoparticles in **(A)** Conjugation of drugs like cetuximab *via* a PEG linker **(B)** Non-invasive MRIs *via* Heparin coated iron oxide nanoparticles and **(C)** Coating them with polymers like PEG for drug delivery purposes. This image was adapted from (Chenthamara et al., 2019). This image is reprinted under the terms of the Creative Commons Attribution 4.0. International License).

experiments provided mixed results where general and organ (lung) toxicity was found *in vivo,* and only moderate toxicity levels were detected *in vitro* (Szalay et al., 2012).

Like iron, zinc oxide nanoparticles have also shown potential as antiviral therapeutics against for COVID-19. Zinc is an FDA-approved substance (Hamdi et al., 2021). A recent study combining zinc oxide nanoparticles coated in triptycene organic molecules (TRP) and ellagic acid (ELG) illustrated highly effective antiviral potential for deactivating human influenza and human coronavirus (Abouaitah et al., 2021). Aside from this, there is evidence that zinc oxide nanoparticles are useful as drug delivery systems. Perera et al. (2022) study investigating the efficacy of albumin-grafted, polycaprolactone-coated, zinc oxide-loaded cloxacillin (APCL-CLOX-ZnO) nanoparticles for drug delivery revealed that 70% of the particles were able to reach the alveoli level during the *in vitro* lung deposition. Because SARS-CoV-2 is known for targeting respiratory organs, similar formulations may be beneficial for delivering therapeutic drugs to patients. Another study analyzing the drug releasing properties of zinc oxide nanoparticles when loaded with amoxicillin found that they could generate controlled delivery of the amoxicillin (Palanikumar et al., 2013). This study showcases zinc oxide nanoparticles and its ability to enhance the potency of treatment through targeted delivery of drugs to specific organ systems in addition to being more cost effective. Current research has yet to thoroughly elucidate the effects of zinc oxide nanoparticles on specific SARS-CoV-2 variants.

In terms of its toxicological properties, the hemolytic effects of zinc oxide nanoparticles are known to increase with higher doses. However, only 5% of hemolysis was determined in the tested concentrations according to a 2021 study (Hamdi et al., 2021). When analyzing the cellular uptake of zinc oxide nanoparticles, major reduction of cell viability was illustrated at a concentration close to 100 μg/ml of zinc oxide nanoparticles (Hamdi et al., 2021). While these studies may indicate a low toxic profile for zinc oxide nanoparticles with specific doses, there are other toxicological aspects to consider. For example, the process of preparing zinc oxide may release particles in the air that could be inhaled by those around the site. Inhalation of zinc oxide can induce a condition known as metal fume fever which carries symptoms such as coughing and fatigue (Liu et al., 2016). There is speculation that there may be similarities between metal fume fever and effects of inhaling zinc oxide nanoparticles, therefore, proper protection should be used when working with these inorganic nanomaterials (Liu et al., 2016).

3.2.5.2 Using quantum dots

Recently, the role of quantum dots (semiconductive nanoscale particles) in COVID-19 therapeutics have caught the attention of researchers. Rocha *et al.* proposed the use of hybrid nanoceria (CeO_2) quantum dots composed of nanoceria

and polymeric matrix of microcrystalline cellulose (MCC) (Rocha et al., 2022). Antioxidant properties along with a unique cubic fluorite structure with a vacant oxygen site (allowing redox cycling) makes nanoceria a nanoparticle of interest that possesses the ability to neutralize ROS. Furthermore, this nanoparticle has been known to inhibit TGF-β and NFkB signalling pathways which are both a part of COVID-19 pathogenesis as induced by the release of cytokines like IL-6, IL-8, TNF-α, and TGF- β. This group has performed spectroscopic and microscopic analysis to produce these hybrid quantum dots that could be adapted for clinical applications such as aerosol delivery to neutralize ROS in COVID-19 patients. Another interesting application of quantum dots is modifying carbon quantum dots (CQD) with boronic acid *via* a hydrothermal process (Loczechin et al., 2019; Xue et al., 2022)). Boronic acid inactivates the variant of human coronavirus (HCoV-229E) at an effective inhibition concentration of 52 ±8 ug/ml by interacting with the glycan units on the virus surface. Hence, modifying quantum dots increases their antiviral activity against the virus. Although metal nanoparticles provide several interesting ways to tackle SARS-CoV-2, their toxicity and appropriate dosage needs to be evaluated if these novel approaches reach clinical trials. Besides that, the other challenge associated with metal nanoparticles is keeping their formulation the same by ensuring their thermal and chemical properties remains the same. For instance, when delivering the NPs, they are exposed to external environment which might lead to chemical damage (Kumar et al., 2022) To address the potential toxic impacts of several nanoparticles, recent studies have emerged on organic nanoparticles. Several properties of organic nanoparticles such as non-toxicity, site-specificity, and biocompatibility make them attractive for COVID-19 therapeutics (Kerry et al., 2019). Lee et al. (2021) proposed a DNase-1 coated polydopamine-poly (ethylene glycol) or polydopamine-PEG to counteract tissue damage caused by cytokine storm or neutrophil activities (production of neutrophil extracellular trap or NETs) in patients infected with SARS-CoV-2. The *in vivo* studies performed also showed inhibition of NF-κB and cytokines.

4 Disease modelling of SARS-CoV-2 as a future avenue for optimizing nanotechnologies for vaccine and therapeutic development

Vaccines are typically expected to reach the market in around 5–18 years and production of these solutions for distribution or public use addsadditional months to years. During the pre-clinical stages of vaccine development, the primary objective is to assess the safety and immunogenicity in cell culture or animal disease models to determine a starting dose (Plotkin et al., 2017). Although the development of mRNA vaccines to address COVID-19 were completed in record-breaking timelines compared to traditional vaccine developments (Ball, 2021), there is still room for accelerating processes to get candidates to successfully enter phase I vaccine trials. One way to accelerate vaccine development in the context of SARS-CoV-2 and its VOCs is to design effective disease models. In doing so, potential nanotechnology-based vaccines and therapeutics can be screened in a cost-effective manner prompting for reduction in animal use, as well as customizability to screen on different patient-specific models including those in vulnerable populations like the pregnant and the elderly.

While two-dimensional (2D) tissue models and animal models allow for a characterization in cell phenotype, physiology, and function, the 2D environment does not replicate the entire complexity of interactions or behaviour of cells *in vivo*. 3D models can potentially mimic the natural extracellular matrix (ECM) of a specific cell type prompting for better understanding of spatial limitations, mechanical properties, and delivery of essential factors required for growth (Duval et al., 2017). Since the onset of SARS-CoV-2, research groups have developed three-dimensional (3D) models from organoids to 3D bioprinted constructs to model the effect of SARS-CoV-2 infection in several tissue types. The most common tissues utilized to investigate SARS-CoV-2 infection contain cells from the respiratory system (lung models), cardiovascular system (heart models), and nervous system (brain models). In this section, we outline current disease models of SARS-CoV-2 and highlight their potential to screen nanomaterial-based vaccines and therapeutics.

4.1 Respiratory models for studying COVID-19

Research on 3D organoid models for SARS-CoV-2 infection often focuses on human alveolar cell types or the respiratory epithelium cell layer to detail the extent of viral infection, progression of infection, and potential immune responses. Youk et al., 2020 illustrates phenotypic changes alongside increased expression of interferon and proinflammatory genes that occur following SARS-CoV-2 infection in human lung alveolar type 2 cells self-organized in a 3D fashion (Youk et al., 2020). Similarly, Lamers et al. (2021) organoid-derived bronchioalveolar model comprising of alveolar, basal, and rare neuroendrocrine cells grown in 3D culture reveal consistent interferon responses against SARS-CoV-2 infection. Application of these 3D lung epithelium models have been utilized to study COVID-19 variants. In fact, experiments on respiratory epithelium organoids alongside designed 2D airway organoid, conducted by Chiu et al., 2022, have successfully modelled transmissibility and infectivity of the SARS-CoV-2 Omicron variant *in vitro* (Chiu et al., 2022*)*. These respiratory models have also been utilized for drug screening potential therapeutics. For example, Wu et al. (2022) paper

utilized type 2 alveolar epithelial cells (AT2) cultured in 3D spheres as a distal lung model to screen the ability of protease inhibitor Camostat Mesylate in blocking viral entry of SARS-CoV-2. Other respiratory models comprise of 3D synthetic hydrogel-based culture systems as an infection platform to test nanoparticle decoys mechanisms against SARS-CoV-2 (Pennarossa et al., 2021). Such models provide insight into the biology of COVID-19 and can serve a tool for drug screening.

4.2 Cardiovascular models for studying COVID-19

Given evidence of SARS-CoV-2 inducing cytotoxic effects on human cardiomyocytes (Bojkova et al., 2020), and the observed rare cases of myocarditis (inflammation of the heart muscle or myocardium) or pericarditis (swelling of the pericardium) after COVID-19 vaccination (Diaz et al., 2021) researchers have worked toward modelling the effects of SARS-CoV-2 in heart tissues to elucidate the mechanisms that lead to the inflammation. One study conducted by Bailey et al., 2021 infected SARS-CoV-2 into engineered heart tissue models used human myocardial cells to highlight the correlation between cardiomyocyte infection and COVID-19 myocarditis as supported by evidence of innate immune response activation *via* RNA sequencing (Bailey et al., 2021). This study also serves as evidence of decreased cardiomyocyte contractility, increased sarcomere breakdown and cardiomyocyte apoptosis resulting from SARS-CoV-2 infections. Anti-inflammatory therapeutics specific to COVID-19 have yet to be tested on these platforms.

4.3 Nervous system (brain) models for studying COVID-19 disease

For studies that investigate the mechanisms of nervous system infection or neurotropism of SARS-CoV-2, 3D organoid models and 3D bioprinted models have been introduced. Experiments conducted by Yi et al., 2020 elucidates the infectious properties of spike protein-pseudotyped SARS-CoV-2 on ACE2 receptor expressing cortical neurons within a 3D forebrain organoid model to quantify the virus's multiplicity of infection (MOI). As a result, the studies imply that the degree of neural infectivity is independent of viral load further validating the use of their 3D organoid models (Yi et al., 2020). Researchers have applied 3D bioprinting techniques in combination with tunable scaffold based, cell-laden, bioinks to produce 3D bioprinted neural tissue models to model neurotropism to mimic the *in vivo* extracellular matrix of neural tissues for enhanced infectious modelling of SARS-CoV-2.3D Bioprinting allows for precise placement of cells alongside components of its extracellular matrix to support the cells leading to better modelling of cellular behaviours *in vitro*. A key example of this development is exemplified by researchers de Melo et al., 2022, where they infected a 3D bioprinted neural-tissue like model consisting of murine astrocytes in a gelatin, GelMa, and fibrinogen-based bioink with mouse-adapted SARS-CoV-2. In observing their 3D bioprinted model, this paper validates the potential of 3D bioprinting techniques for disease modelling of infectious diseases especially in tissue areas that are challenging to study (de Melo et al., 2022).

In conclusion, several respiratory, cardiovascular, and nervous system disease models have been thoroughly made to SARS-CoV-2 infection yet there is a limited number of studies that have applied mRNA-LNP vaccine technologies and other nanotechnology-based therapeutics to these models. Although these models are preliminary in that they do not entirely demonstrate the adaptive immunity mechanisms and some generally utilize a single cell type as opposed to cocultures of various cell types, innate immunity mechanisms would be helpful in further characterizing nanotechnology-based behaviours within the human body.

5 Conclusion

Ultimately, the use of nanomaterials shows great promise for addressing COVID-19 through future advancements in vaccines and therapeutics. However, further research must be made to these treatments to fully optimize their potentials in healthcare. As previously stated, evidence illustrates that mRNA vaccines using lipid nanoparticles can be highly advantageous compared to conventional formulations as they are cost-effective, can be rapidly produced, and carry self-adjuvanting properties. mRNA is also flexible for sequence modifications which aids in addressing variants. Despite these benefits, there is still speculation on the longevity and physiological effects of LNPs. A previously mentioned study illustrated a substantial decrease in immunity 6 months after two doses of the Pfizer vaccine. This may be linked to mRNA impurities caused by the ionized lipids disrupting mRNA translation. This is an indicator that the implementation of annual boosters may be necessary for maintaining immunity against the virus and new variants. Given the fast rate of production, flexible sequences, and cost-effectiveness of these vaccines, they may be good candidates for large scale, annual production. In addition, adverse effects of mRNA vaccines such as fever, fatigue, and hypersensitivity may be triggered using LNPs. To illustrate, research found that LNPs caused intense inflammation in mice which may cause symptoms and that the PEGylated (polyethylene-glycol) lipid component can stimulate immune responses leading to hypersensitivity.

In terms of therapeutics, the utilization of nanoparticles alongside currently available treatments has shown promise in minimizing drug toxicity and bioavailability through drug

delivery systems. There are also nanomaterials that have merit in their antiviral properties. In a study analyzing monoclonal antibody (mAb) production using modified mRNA encapsulated in LNPs (RBD mRNA-LNPs), found the mAbs produced by immunizing mice with RBD mRNA-LNPs had potent viral neutralizing abilities against the Alpha and Delta variants (Hsu et al., 2022). Unfortunately, there is very limited research detailing the efficacy of nanotechnological therapies against COVID-19 variants of concern and ways of addressing potential toxicological effects of these particles. For example, a previously stated study indicated that zinc oxide nanoparticles may work as an antiviral therapeutic against COVID-19, but for implementation into medical practice, potential toxic properties of the particles such as hemolysis occurring upon certain doses must be further studied and addressed.

Considering the known benefits and obstacles of nanoparticles in COVID-19 treatments, the medical advancements and knowledge gained through the pandemic can be significantly helpful in improving upon current medicines and aid in production of treatments against other viruses. For example, a study using chimeric spike mRNA vaccines in mice demonstrated that the vaccines were able to neutralize various SARS-like zoonotic corona viruses (Martinez et al., 2021). Considering how mRNA vaccines are currently being used for preventing COVID-19, this work proves that technology used for combating COVID-19 may be used and adjusted for other viruses. With this in mind, we can foresee future advancements in medicine utilizing nanotechnological strategies devised during the pandemic to aid in preventing and treating other illnesses.

Author contributions

SW conceptualized contents of the paper, gathered references, and guided the group in synthesizing the paper at each stage, including revisions of the manuscript. MVH created an outline for the paper based on SW's guidance, wrote Section 4 entirely and parts of Section 2 along with gathering references, edited drafts, organized references, formatted final manuscript/figures. MVH organized references, wrote the introduction, conclusion, and Section 4.2; assisted with edits. NK gathered/organized references for their written contributions; Sections 2.1–2.3. JWS gathered/organized references for their written contributions; Section 2 introduction, Sections 2.2–2.3. SK gathered/organized references, wrote Section 3.2 and acknowledgments section, completed edits for their section. CV gathered/organized references, wrote Section 3.1, completed edits for their section. DP for gathered references and written portions of Section 2.1.

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Conflict of interest

SW is the C.E.O. of Axolotl Biosciences a start-up focused on selling novel bioinks for bioprinting human tissue models.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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COVID-19 repellent cloth

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In this research work, for the first time, we have developed and demonstrated a COVID-19 repellent coating on cotton cloth that not only repels the virus but also most of the human body fluids (superhemophobic). The coating was tested in the BSL3 lab. The controlled experiments revealed no significant increase in the log viral particles on coated fabric compared to the uncoated surface, evidence that the coated fabric resisted the SARS-CoV-2 inoculum. Further, the coated cloth exhibited excellent dust-free nature and stain resistance against body fluids (blood, urine, bovine serum, water, and saliva aerosol). It also shows sufficient robustness for repetitive usage. The fabrication process for the developed COVID-19 repellent cloth is simple and affordable and can be easily scaled up for mass production. Such coating could be applied on various surfaces, including daily clothes, masks, medical clothes, curtains, etc. The present finding could be a mammoth step towards controlling infection spread, including COVID-19.

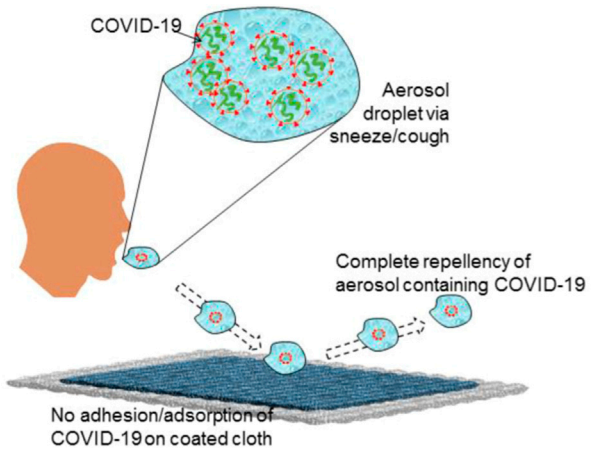
KEYWORDS

COVID-19, SARS-CoV-2, anti-viral, superhemophobic, virucidal

Introduction

In late 2019, a new virus, SARS-CoV-2 (also known as COVID-19) emerged. Within a few months of emerging, it created a havoc situation worldwide, and eventually, WHO declared it a pandemic in March 2020 (Cucinotta and Vanelli, 2020). Due to its highly transmissible nature, nearly all nations reported infection cases from COVID-19. Most infected patients experience mild to moderate respiratory illness, mainly common flu symptoms, and recover with standard flu treatment. However, some people with medical history such as diabetes, chronic respiratory disease, and cardiovascular disease may also experience severe illness or death. It is estimated that COVID-19 has resulted in more than 6.5 million deaths and billions of dollars in losses (Who, 2020b). Infection of the virus occurs through the respiratory system *via* the direct transmission of airborne droplets (sneeze, cough), direct contact transmission (blood or other liquid, skin), indirect transmission from a surface (respirator mask, cloth, carpet, handles, switches, and table).

PPEs are not 100% safe as the virus remains alive on them for many days and requires very careful disposal practices to avoid further infection spread. Besides this, people face a tough time working with wearing these single-use multilayer full-body cover PPEs. As these PPEs are uncomfortable and expensive, most people bypass the protocols.



GRAPHICAL ABSTRACT

Therefore, if COVID-19-repellent affordable PPEs are developed, which can be used multiple times, this will help to control the spread of infection to a huge extent.

The viral-repellent coating can be done by designing a surface with an excellent anti-wetting state that comprises a high static contact angle and low tilt angle with body fluid (blood, urine, protein, saliva aerosol, and water) (Galante et al., 2020). Typically, such a surface comprises nano and micro rough structures trapping air which makes a liquid-air interface and leads to superhemophobicity (blood static contact angle greater than 150° and tilt angle less than 10°). Designing a superhemophobic surface is more challenging as blood has a much lower surface tension (about 54 mN/m) (Tang and Hu, 2005; Leszczak et al., 2013). Body fluid is a direct carrier of transport viruses, microbes, and pathogens that lead to people associated with infected patients.

In view of the above findings, we have developed a novel COVID-19 repellent coating on cloth *via* an easy and economical surface modification method. This coated cloth is COVID-19 repellent in nature and was tested against COVID-19 virus in BSL-3, which confirmed excellent COVID-19 repellency. There are proposals on COVID-19 repellency earlier; however, the present study is the first to demonstrate it in reality.

Experimental details

To develop COVID-19-repellent coated cloth, modification of the cloth surface was done by using an immersion technique. Before coating, the as-received cloth was cleaned in water and acetone mixture solution in ultrasonication for 30 min and dried in a hot air oven at a temperature of 60°C. Later on, the coating solution was prepared by adding a 1% w/v of pefluorodecltriethoxysilane (PFDTES, > 97%, procured from Sigma-Aldrich Co., Ltd) into 40 ml ethanol (procured from Changshu Hongsheng Fine Chemical Co. Ltd.) and stirred for 2 h at the room temperature. The clean and dried cloth was immersed in the prepared coating solution for 24 h. Then, the sample was taken out and dried in a hot air oven at 90°C for 2 h.

The coated and uncoated surfaces were tested against the SARS-CoV2 virus (Indian/a3i clade/2020 isolate) in the BSL3 lab of CSIR-CCMB (ASTM E1052-20, 2020) for 30 and 60 min of contact with the virus. Briefly, the coated and uncoated cloth surface was co-incubated with the virus inoculum for 30 and 60 min, respectively. After the specified incubation time, the virus inoculum (0.1 MOI) from both surfaces was added to the Vero cells and left for 3 h post-infection, the viral inoculum was replaced with fresh media containing 10% FBS and was maintained at 37°C, 5% CO_2, until 72 h. After 72 h, the cell supernatant was collected and spun for 10 min at 6,000 g to remove cell debris, and the supernatant was transferred to fresh collection tubes. The viral RNA was extracted using MagMAX™ Viral/Pathogen Extraction Kit (Applied Biosystems, Thermofisher Scientific), and an automated RNA extraction machine (KingFisher Flex (version 1.01, Thermofisher Scientific) was used as instructed by the manufacturer. The SARS-CoV2 viral copies were detected using COVID-19 RT-q PCR Detection Kit (Fosun 2019-nCoV qPCR, Shanghai Fosun Long March Medical Science Co. Ltd.) following the manufacturer's instructions (Baller et al., 2020). The C_t values of the N gene, E gene, and ORF 1 ab were considered to estimate the % viral reduction and log viral particles.

The liquid (blood, urine, protein, water) contact angle measurements were performed using Tensiometer (DSA 25, Kruss, Germany) by placing a droplet of 3–5 µl on uncoated and coated samples at room temperature. The experiment was repeated at five different locations with each sample to calculate the average value. The surface morphology of the samples was

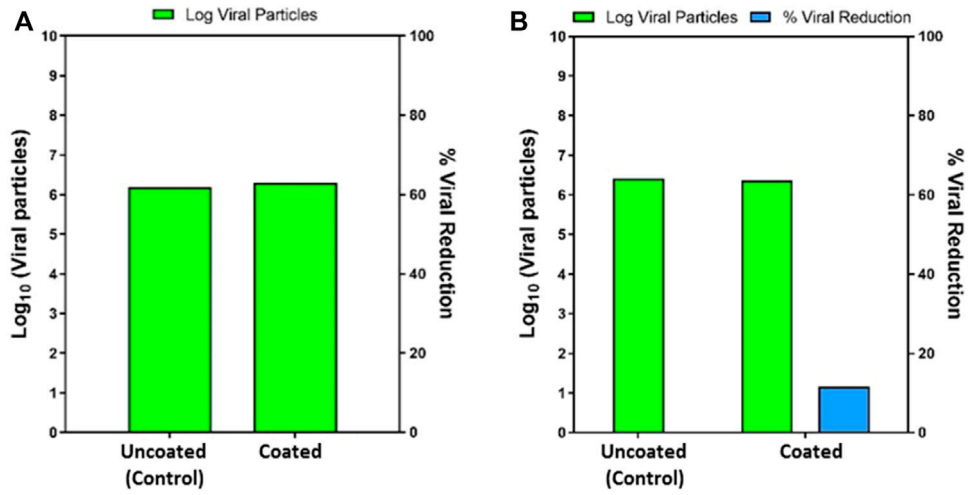


FIGURE 1
(A) The log viral particles at 30 min exposure on uncoated and coated surface (left). **(B)** The log viral particles at 60 min exposure on an uncoated and coated surface (right).

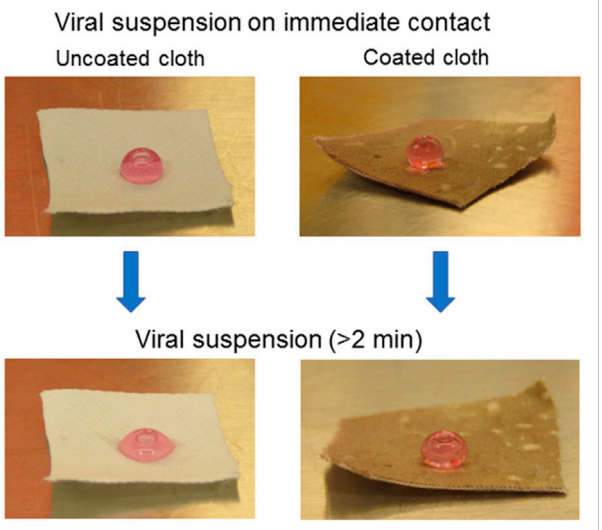


FIGURE 2
The optical images of viral suspension on immediate contact and after some time (>2 min) on uncoated and coated cloth.

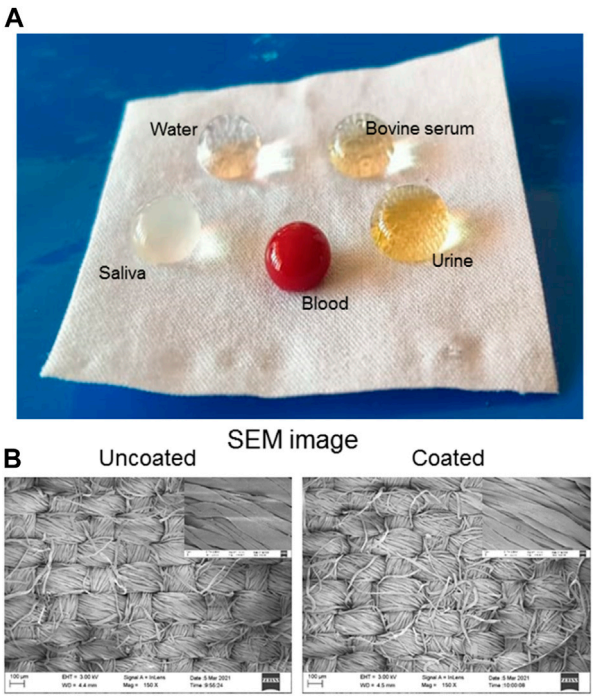
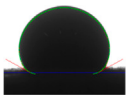
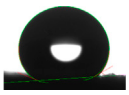
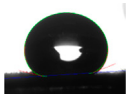
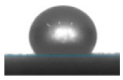
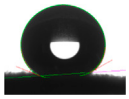


FIGURE 3
(A) Optical image of coated cloth surface containing blood, urine, bovine serum, saliva, and water droplets. **(B)** SEM images of uncoated and coated cloth. Right corner SEM images corresponding to the high magnification.

carried out using a scanning electron microscope (SEM, JEOL, JSM-6480LV). The elemental analysis of the samples was performed using X-ray Photo-Electron Spectroscopy (XPS) with Auger Electron Spectroscopy (AES) module and C60 sputter gun (PHI 5000 Versa Probe II, FEI Inc.).

In the self-cleaning experiment, uncoated and coated samples were forcefully dipped into the muddy water for 10 min using the tweezer. Later, the samples were removed from the muddy water, and the behavior of the muddy water droplets was studied on the surfaces. The stain resistance

TABLE 1 The contact angle of body fluid for uncoated and coated cloth surfaces and their images.

Body fluid	Surface tension (mN/m)	Contact angle (°)		Droplet image showing contact angle of coated cloth
		Uncoated	Coated	
Blood	55.89 ± 3.57 (Hrncir and Rosina, 1997)	~0	155	
Urine	50–60 (Thomas et al., 2009)	~0	154	
Bovine serum	<72 (Le et al., 2022)	~0	162	
Saliva aerosol	55–65 (Gittings et al., 2015)	~0	157	
Water	72 (Le et al., 2022)	~0	155	

experiment was performed by placing the liquid (urine and blood) droplets on uncoated and coated samples and later drying them by keeping the samples on the heating plate at 60°C for a few minutes. The urine and bloodstains left on the coated and uncoated surfaces were studied later.

The water absorption capacity of the uncoated and coated samples was measured. Initially, all the samples were immersed in water for 10 min. After draining the excess water, the weight of the samples (w_a) was measured and compared with the weight of the sample before immersion in water (w_d). The absorption capacity of the samples was calculated using Eq. 1.

$$\text{Water absorption capacity } (\%) = \frac{w_a - w_d}{w_d} X100 \qquad (1)$$

Results and discussion

In this study, COVID-19 repellent cloth was developed by immersing cloth samples in PFDTES solution.

In virus testing, there was no significant reduction observed in the log viral number on exposing the viral inoculum on the coated and uncoated surface for 30 and 60 min (Figure 1). This may be due to the liquid-repellent nature of the coated surface. The contact of viral particles on the coated material was eliminated, and no viral increase was observed. This data proves the repellant nature of the test surface's viral suspension repellent nature and supports the intactness of the droplet containing viral particles over the tested time (Figure 2).

The surface wettability of coated cloth was further measured and compared with the uncoated cloth. The cotton fabric exhibits superhemophilic behavior with a blood static contact angle of ~0°. The blood repellency of coated cloth is measured at a static contact angle of 155°, indicating that the fabric has attained superhemophobicity. Additionally, body fluids (urine, protein, saliva aerosol, and water) also show good repellency with contact angles of more than 150° (Figure 3A, Table 1). The Supplementary Video shows the body fluid's behavior on the coated surface (Supplementary Video S1).

The surface morphology of the cloth surface after coating was examined by SEM analysis, as shown in Figure 3B. After coating, no change in morphology is observed due to the formation of a monolayer on its surface. The XPS results in Figure 4 confirm the chemical compositions of uncoated and coated samples. The XPS survey (Figure 4A) scan of uncoated samples confirms C, O, and Si of cellulosic structure and SiO_2 as textile impurities. For coated cloth, C, O, and Si of cloth and PFDTS, and F of PFDTS are identified. The high-resolution core-level C 1s spectra (Figure 4B) of the coated and uncoated samples are easily distinguishable. The uncoated cloth's C1s spectra exhibits three major peaks at 284.2, 285.7, and 287.7 eV corresponding -C-H/C-C, C-OH, and C-O-C functionalities confirm the cellulosic structure (Wang et al., 2020). The C 1s spectrum of coated cloth exhibit six major peaks at 284.1, 285.7, 287.1, 287.8, 290.8, and 293.1 eV corresponding to C-H/C-C, C-OH/C-O-Si, C-Si, C-OC, -CF_2, and -CF_3 functionalities confirms

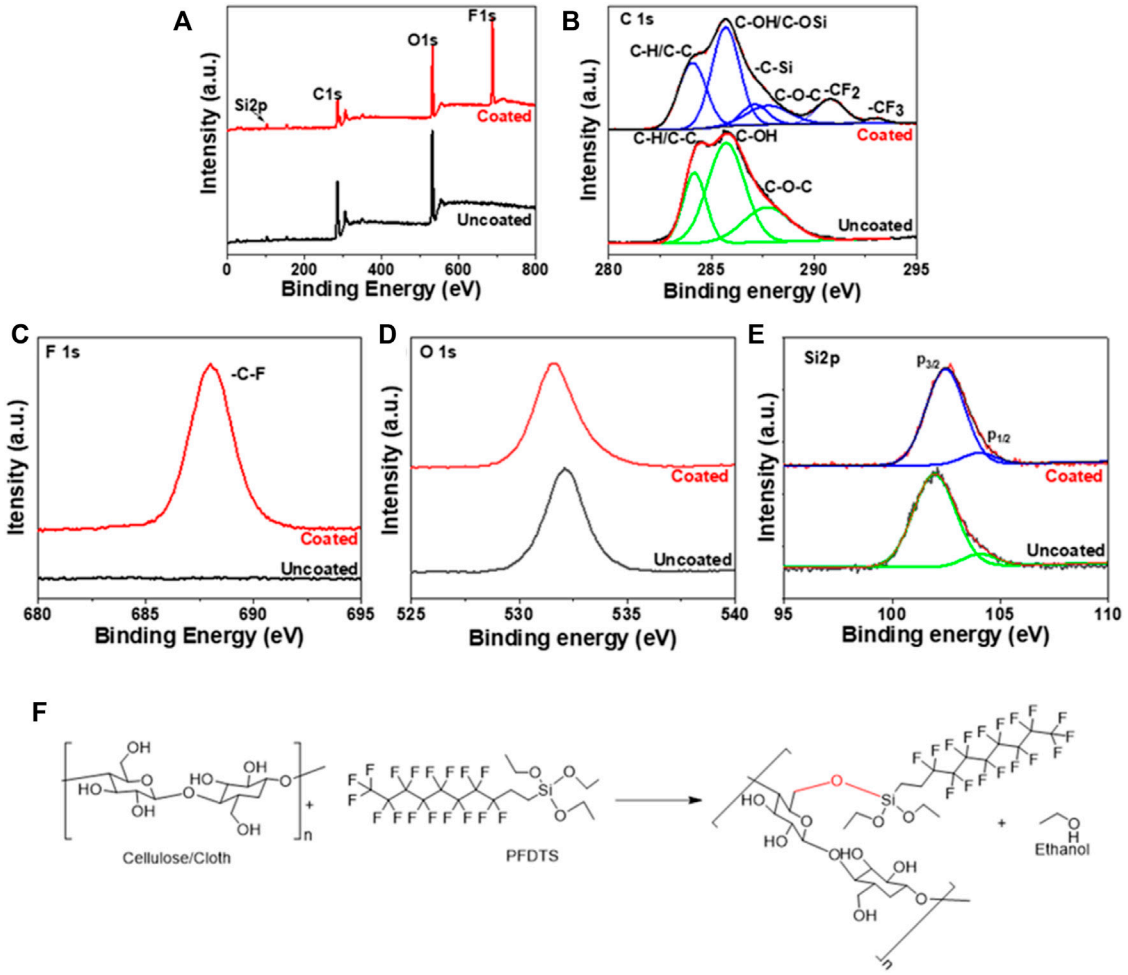


FIGURE 4
(A) Survey **(B)** C1s **(C)** F1s **(D)** O1s, and **(E)** Si2p XPS spectra of uncoated and coated cloth. **(F)** Mechanistic pathway of PFDTS coating on cloth.

the presence of PFDTS and cellulosic structure (Korolkov et al., 2020). The high-resolution F1s spectra (Figure 4C) of coated cloth exhibits a peak at 688.0 eV corresponding to C-F functionality (Korolkov et al., 2020). The high-resolution O1s (Figure 4D) reveals a prominent peck shifting for coated (531.6 eV) and uncoated (532.2 eV) samples indicating a significant change in the oxygen chemical environment (Korolkov et al., 2020; Wang et al., 2020). Besides the carbon-oxygen bond of cellulose in the coated sample, oxygen is also bonded with silicon. The high-resolution Si 2p spectra of samples are presented in Figure 4E. The spectrum of uncoated cloth reveals peaks at 101.9 and 104.0 eV for 2p3/2 and 2p1/2 electrons corresponding to SiO_2 impurities often found in textiles. The spectrum of coated cloth exhibits peaks at 102.4 and 104.0 eV for 2p3/2 and 2p1/2 electrons corresponding to the silane group of PFDTS (Korolkov et al., 2020). It is also observed that the Si content increases in coated cloth compared to uncoated cloth. The observed XPS data not only confirms the PFDTS but also indicates the (PFDTS) Si-O-C (cellulose) interaction leading to a stable PFDTS coating on the cloth surface. A probable scheme is presented in Figure 2F displaying the coating formation mechanism.

The ability of a coated cloth to sustain against the dirt is demonstrated in Figure 5. For the experiment, uncoated and coated samples were immersed in a mud water solution for 10 min. It is noted that the coated fabric floats over the muddy solution due to its water repellency and is difficult to immerse; so, it is forcefully immersed in the solution. After 10 min immersion, the uncoated surfaces were soaked with muddy water, whereas coated cloth remained clean, indicating that mud particles do not adhere to the coated surface due to the air-solid interface present on the surface.

Body fluid (mainly blood and urine) leaves stain marks on the medical clothes/devices, including the apron, masks, bedsheets, and covers due to the inherent hydrophilic nature of the cloth fabric surface. In most cases, these stain marks do not

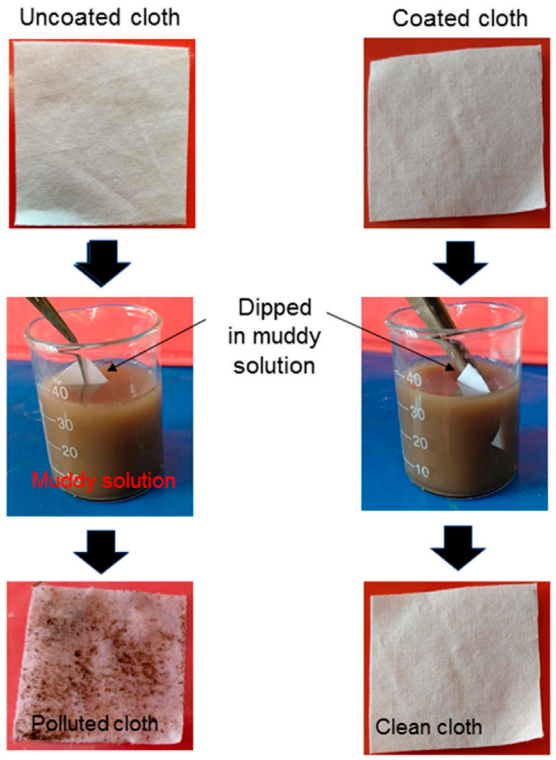


FIGURE 5
Optical images of uncoated and coated cloth, before and after immersing in the muddy solution, showing the ability of a coated cloth to sustain against the mud.

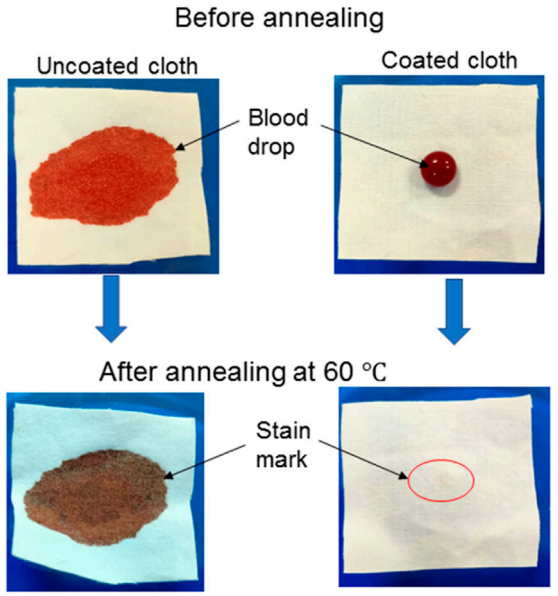


FIGURE 6
The optical images of blood droplets before and after drying on uncoated cloth (left the stain mark) and coated cloth (exhibit negligible stain marks), showing the stain-resistant ability of both coated cloths.

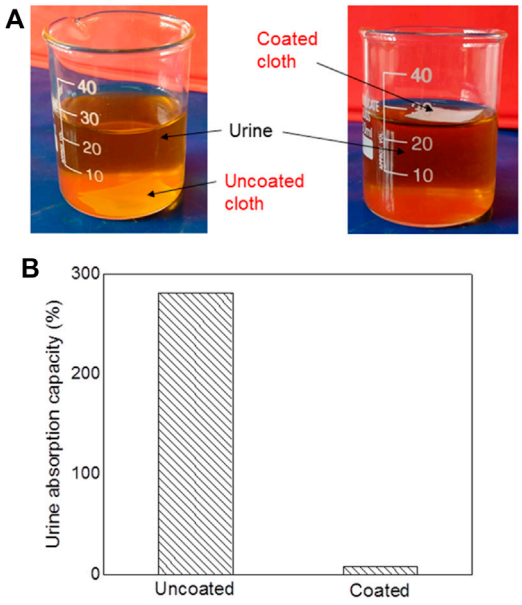


FIGURE 7
(A) The optical images of uncoated and coated cloth immersed in urine for 10 min, show the urine absorption ability. **(B)** Results of urine absorption capacity for uncoated and both coated cloths.

fade away even after rigorous washing. In consideration of this, we have performed anti-stain and liquid absorption tests. Results of the anti-stain property of cloth before and after coating demonstrated that the blood droplets are entirely absorbed by uncoated cloth and spread over it, whereas these droplets make spherical shapes, as shown in Figure 6. A similar observation was seen for colored water and urine. Later these droplets containing samples were kept in a hot air oven at 50°C. After evaporation, these fluids leave stain marks on the uncoated cloth surface. Whereas there are negligible stain marks on a coated cloth surface, showing its anti-stain property.

In the liquid absorption test, the uncoated and coated cloth was immersed in urine, as shown Figure 7. Initially, the weight of all samples was 0.043 g. After immersion, it was 0.167 and 0.047 g for uncoated and coated cloth, respectively. The calculated urine absorption capacity of cloth is shown in Figure 7. The uncoated cloth shows high urine absorption of 281% which is attributed to the cloth's inherent superhydrophilic characteristic. The coated cloth exhibits a urine absorption of 8%, which is only 2.8% of the uncoated cloth. This result reveals that the urine absorbing capability is higher in uncoated samples compared to coated samples. The coated samples also show small urine absorption due to the non-homogeneity coating on its edges side.

The global supplies for PPEs and masks due to their regular use are a constraint and make it harder for industries to meet the requirements. Incorporating thermal disinfection, UV-Illumination, or hydrogen peroxide vapor exposure (Celina et al., 2020) is a known time-consuming disinfection method. The present coating reduces the need for the aforementioned sanitization processes to a simple one, such as cleaning with a non-reactive disinfectant to the coating for 99% elimination of the viral particles.

Conclusion

In this paper, we have demonstrated a facile and durable COVID-19 repellent coating on cloth fabric. Results of the coated cloth showed the complete repellency of body fluid (blood, urine, bovine serum, water, saliva aerosol) with a static contact angle greater than 150°. Coated cloth showed non-adherence to dirt, stains from the body fluid, and negligible body fluid adsorption. The interaction of COVID-19 viruses with uncoated and coated was examined at BSL-3 research facilities, and it was found that coated cloth showed complete repellency to the viruses. Experimentally coated cloth showed good washing durability, i.e., it can be used multiple times. Since this COVID-19 repellent-coated cloth is made by a straightforward immersion technique, scaling up at the industrial level will be easily feasible. Commercializing such a product for general public use at economical rates will really help fight against the COVID-19 pandemic.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The SARS CoV2 study was approved by the Institutional Bio-safety Committee of CSIR Center for Cellular and Molecular Biology, Hyderabad, India.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Application of nanomaterials against SARS-CoV-2: An emphasis on their usefulness against emerging variants of concern

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Researchers are now looking to nanomaterials to fight serious infectious diseases that cause outbreaks and even pandemics. SARS-CoV-2 brought chaos to almost every walk of life in the past 2 years and has challenged every available treatment method. Although vaccines were developed in no time against it, the most pressing issue was the emergence of variants of concern arising because of the rapidly evolving viral strains. The higher pathogenicity and, in turn, the higher mortality rate of infections caused by these variants renders the existing vaccines less effective and the effort to produce further vaccines a costly endeavor. While several techniques, such as immunotherapy and repurposed pharmaceutical research, are being studied to minimize viral infection, the fundamentals of nanotechnology must also be considered to enhance the anti-SARS-CoV-2 efforts. For instance, silver nanoparticles (AgNPs) have been applied against SARS-CoV-2 effectively. Similarly, nanomaterials have been tested in masks, gloves, and disinfectants to aid in controlling SARS-CoV-2. Nanotechnology has also contributed to diagnoses such as rapid and accurate detection and treatment such as the delivery of mRNA vaccines and other antiviral agents into the body. The development of polymeric nanoparticles has been dubbed a strategy of choice over traditional drugs because of their tunable release kinetics, specificity, and multimodal drug composition. Our article explores the potential of nanomaterials in managing the variants of concern. This will be achieved by highlighting the inherent ability of nanomaterials to act against the virus on fronts such as inhibition of SARS-CoV-2 entry, inhibition of RNA replication in SARS-CoV-2, and finally, inhibition of their release. In this review, a detailed discussion on the potential of nanomaterials in these areas will be tallied with their potential against the current and emerging future variants of concern.

KEYWORDS

SARS-CoV-2, variants of concern (VOCs), COVID-19, nanomaterial, nanotechnology, mutations

1 Introduction

The history of humankind has witnessed more than 15 pandemics and numerous outbreaks since the earliest recorded pandemic during the Peloponnesian war in 430 B.C. Some of the most recent epidemics were severe acute respiratory syndrome (SARS), Middle East Respiratory Syndrome (MERS), and Ebola (Lombardi et al., 2021). In late 2019, a high number of patients with pneumonia of unknown cause were reported in Wuhan city of China. They soon confirmed a novel coronavirus, "SARS-CoV-2 caused it." SARS-CoV-2 was revealed to be phylogenetically associated with SARS viruses according to its genomic analysis (Rume and Islam, 2020). It was declared a "public health emergency of international concern" by WHO on 30 January 2020, and only 39 days later, on 11 March 2020, they declared the COVID-19 pandemic (Tarkar and Technology, 2020). The general symptoms of COVID-19 infection are fever, nausea, cough, loss of smell and taste, fatigue, diarrhea, sore throat, and vomiting. Although, the severity of COVID-19 leads to respiratory failure, cardiac injury, and even death (Struyf et al., 2022). As of 1 November 2022, there have been 635,472,882 confirmed cases of the coronavirus disease (COVID-19), including 6,593,723 deaths, according to the world health organization (WHO) (worldometer, 2022). Vaccination has always been the most effective strategy for controlling epidemics and outbreaks (Cleve, 2021). To tackle the disease, companies and organizations worldwide have attempted to develop COVID-19 vaccines to achieve herd immunity since the start of the pandemic (Haynes et al., 2020). Different techniques have been employed to create vaccines against SARS-CoV-2 (Jeyanathan et al., 2020). The approaches to COVID-19 vaccine development include live-attenuated vaccines, adenovirus vectors, inactivated viruses, recombinant protein, and DNA and RNA vaccines. RNA vaccines emerged as a revolution to cope with the pandemic. However, the consistently changing characteristic of the virus is a challenge. SARS-CoV-2 is undergoing rapid changes in its genome and structure, and new variants are constantly resulting. Some of the variants of SAR-CoV-2 are Alpha (emerged in September 2020, United Kingdom), Beta (May 2020, South Africa), Gamma (November 2020), Delta (October 2020), and Omicron (November 2020, many counties). Because more novel SARS-CoV-2 variants are expected, the currently available vaccines, preventive measures, and antiviral drugs are, therefore, only temporarily effective.

Consequently, it is time to investigate rapid and accurate diagnostic tools and therapeutics with higher efficacy. Considering these challenges, experts suggest that nanotechnology can play a pivotal role in facing these challenges and managing COVID-19. Nanotechnology has the potential to overcome the limitations in the existing therapies and strategies used against SARS-CoV-2. Nanotechnology offers different possibilities and approaches to combat the virus. We can develop novel and rapid diagnostic kits and therapeutics through nanotechnology and design more effective drugs and vaccines (Ruiz-Hitzky et al., 2020). The size, shape, surface-to-volume ratio, and other unique physiochemical properties of nanomaterials enrich them with potential for purposes like drug delivery, antibody development, vaccine formulation, and personalized treatment (Paliwal et al., 2020). Nanoparticles, quantum dots, nano-based biosensors, lipid-based nanoparticles, and nano-assemblies of polymers or proteins are the types of nano-systems that have already been employed for the detection, treatment, delivery, and development of vaccines against the virus (Jindal et al., 2017; Alphandéry, 2020). Various metallic nanoparticles can potentially be used for diagnosing and identifying SARS-CoV-2 and other variants of concern (VOCs) (Rashidzadeh et al., 2021). Organic and polymeric nanoparticles play a pivotal role in the drug delivery systems of coronaviruses. Specific ligands can be used to functionalize the antigen-loaded nanoparticles. For instance, Raghuwanshi et al. (2012) developed plasmid DNA-loaded chitosan nanoparticles and used them as a vaccine for different variants of SARS. Literature reviews have already shown the immense potential of nanotechnology in coping with pandemics like COVID-19 (Yasamineh et al., 2022). Detailed studies are available that report the potential of nanotechnology against SARS-CoV-2 (Sahu et al., 2021). There are, however, limited studies on how nanotechnology can contribute towards the fight against variants of SARS-CoV-2 to reduce the burden and stop the pandemic quickly. For instance, Huang X. et al. (2022) have reported the use of nanotechnology-based strategies against VOCs. However, most of this study is focused on nanoparticle-based antibody eliciting vaccines. An account of the use of nanomaterials especially metallic nanoparticles at the front of prevention, treatment and its future implications was needed. The main aim was to emphasize the role of nanomaterials synthesized through different means and possessing potential of preventing VOCs. Therefore, in this review, we will explore the efforts of nanomaterials, including metallic nanoparticles, prevent and tackle SARS-CoV-2. We will mainly focus on the potential of nanotechnology and nanomaterials to cope with variants of SARS-CoV-2 and its future concerns.

2 Using nanotechnology against SARS-CoV-2; an overview

During any outbreak, vaccination has always been the best weapon. While analyzing the previous outbreaks, vaccination has proved a convenient tool that has always been practiced in achieving herd immunity. Through vaccination, the susceptibility of an individual to viral infection is reduced. In addition, it also minimizes the transmissibility of infections

among a population (Gellin et al., 2001). Until the availability of vaccines, therapeutic drugs were used as a temporary solution. In therapeutic strategy, antimicrobial drugs, i.e., antivirals, are used to eliminate the virus from the body. These drugs kill the virus or inhibit its growth or other aspects of its life cycle. Both these approaches play a crucial role in combating any viral strain. However, they have certain challenges to face. For example, in the case of vaccination, new viral strains are always imminent (Le Page, 2021). To cope with the emergence of new strains, it is always necessary to predict their emergence, and be ready to develop new vaccines every few years, and sometimes every year. At the same time, strict patient compliance is an essential condition for the effectiveness of therapeutics. Therapeutic drugs against viral infections may have harsh side effects that sometimes outweigh the benefits. Both strategies demand a lot of resources and time, which are very expensive in emergencies such as pandemics.

Nanotechnology has the potential to overcome these limitations. Nanotechnology has attracted the attention of the medical science community and offers advances in the diagnosis, prevention, and treatment of diseases such as COVID-19. It provides opportunities in the shape of nanomedicines and nanomaterials to tackle the disease. Nanomaterials have been studied widely over the past few decades for their size, shape, and surface-to-volume ratio. These properties make them efficient for the rapid identification and detection of SARS-CoV-2, the design of proper personal protective equipment (PPE), the creation of vaccines, and the treatment of the infection. The food and drug administration (FDA) of the United States has approved 49 antigen diagnostic devices for COVID-19, most of which are based on lateral flow assays (LFA) using either gold nanoparticles (AuNPs) or quantum dots (Xu et al., 2022). AuNPs based-biosensors were recently designed to detect SARS-CoV-2 that can bind to lab-designed DNA receptors rapidly. This technique is very sensitive as SARS-CoV-2 has single-stranded RNA, which readily binds to stabilized complementary DNA receptors (Hasanzadeh et al., 2021). Nanoparticle-based biosensors coupled with highly sensitive loop-mediated isothermal technique (LAMP) are also used for virus detection, and one such biosensor was recently fabricated for SARS-CoV-2 screening. This assay is reliable, less expensive, and highly efficient (Hasanzadeh et al., 2021).

Literature suggests that using nanoparticles to treat COVID-19 is safe and effective for drug encapsulation and toxicity reduction (Lombardo et al., 2019). Chitosan nanoparticles, named Novochizol, possess mucoadhesive properties (Tharayil et al., 2021). Therefore, they can treat the intestinal tract reactions caused by SARS-CoV-2 infection. Chitosan can also encapsulate and carry drugs to the lung to treat severe COVID-19 patients (Cavalcanti and Cajuba de Britto Lira Nogueira, 2020). SARS-CoV-2 can be captured, neutralized, and prevented from infecting host cells using nano-sponges developed by Zhang et al. (2020). These nano-sponges have surface-binding receptors capable of attracting SAR-CoV-2. Although the administration of nanoparticle-based drugs was a complicated issue, that is also addressed. For instance, Itani et al. (2020) suggest the intranasal administration of theranostic nanoparticles to treat COVID-19 as they enable the carrying of therapeutic portions like antibodies.

Nanotechnology also has a pivotal role in the drug delivery systems. Nanoparticles have already been used for this purpose and are still in service. Different therapeutic molecules have been used to deliver antiviral drugs in an inhalable form. However, their poor stability has always been an issue that can be solved by coupling them with different nano-delivery systems. For example, nano-carriers are used to overcome the problem of poorly soluble drugs, which otherwise can cause lung toxicity (Beck-Broichsitter et al., 2012). This means that there is an immense potential for nanotechnology-based strategies in therapeutic applications in case of COVID-19.

Nanotechnology also finds its application in the development of vaccines. Nanovesicles obtained from spike, envelope, and membrane proteins of SARS-CoV-2 have been used as nano vaccines against the virus (Kato et al., 2019). The primary purpose of developing nanoparticle-based vaccines is to provoke an adaptive immune response to generate immunological memory. These memory B cells induced by nanoparticle-based vaccines are expected to live longer than normal vaccine-generated memory cells. This results in antibodies that neutralize the interaction between SARS-CoV-2 and host cell, hence viral clearance (Guerrini et al., 2022). Similarly, lipid-based nanoparticles and peptide-based vaccines for SARS-CoV-2 and some other enveloped viruses have also been employed (Lim et al., 2021). This shows that nanotechnology has the potential to overcome the limitations of traditional strategies for vaccine development against coronavirus infections.

3 Change over time: Mutations in SARS-CoV-2 and the role of nanotechnology

Like other RNA viruses, SARS-CoV-2 also constantly changes through mutation. Generally, due to some selective advantages of the virus, new variants become more common. Whenever a virus replicates itself, structural changes occur to its genome. These characteristic changes of the virus are led by mutations, including characteristics that affect its ability to spread and/or to cause more severe illness and death (Otto et al., 2021). Combining the genetic material from two different variants creates a new recombinant variant. This leads to the creation of variants. More transmissible variants cause severe illness, and those that can easily evade immune response are more public health concerns among many SARS-CoV-2 variants detected (Walensky et al., 2021).

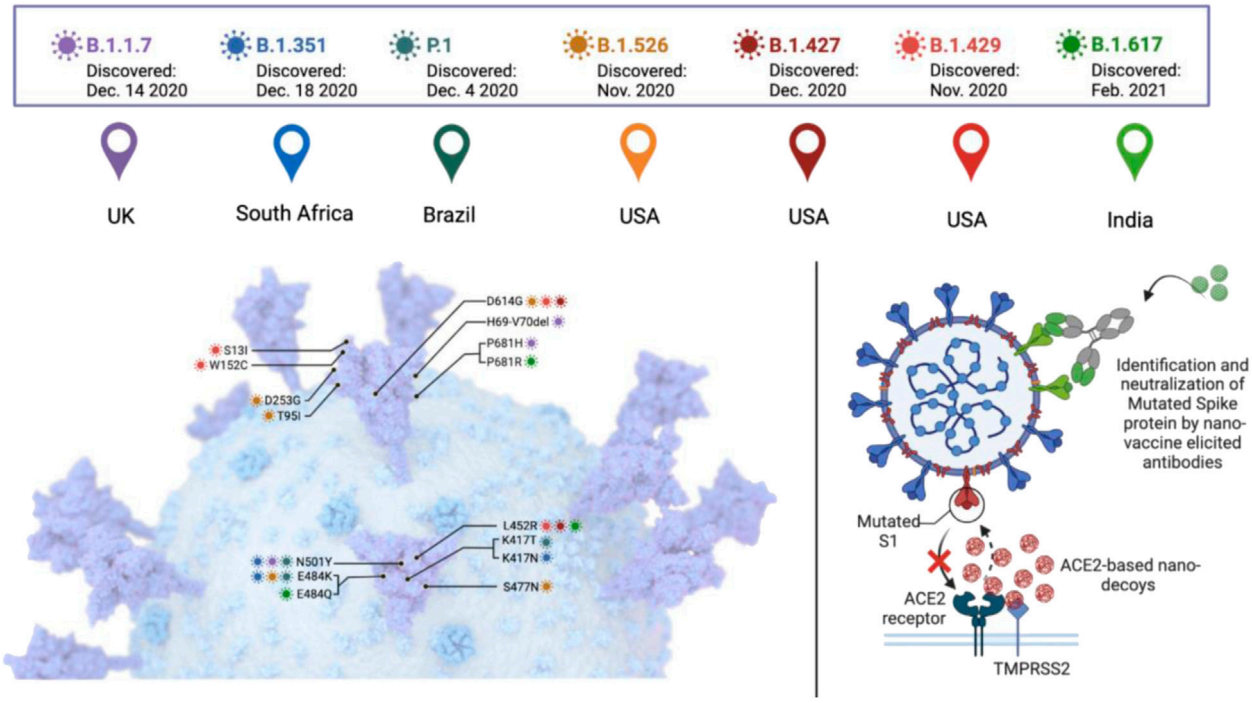

FIGURE 1
A general description of SARS-CoV-2 variants of concern and the potential of nanotechnology in dealing with them.

Since the beginning of the COVID-19 pandemic, the genetic lineages of SARS-CoV-2 have been emerging and circulating the world. In the early stage of pandemics, mutations have been found in the evolved sequence of 103 strains (Awadasseid et al., 2021). The evolutionary rate of SARS-CoV-2 per year has been estimated between 0.0004 and 0.002 mutations per nucleotide. The phylogenetic classification of new lineage emerging SARS-CoV-2 has been difficult because they often differ by just a few nucleotides (Tao et al., 2021). These variants can increase virus transmission rates and reinfection risk and reduce the protection afforded by neutralizing monoclonal antibodies and vaccination.

Different variants of SARS-CoV-2 are grouped into three categories. The first, known as Variants of Interest (VOI), have mutations in binding receptors. These genetic mutations suggest that they are conferred by natural infection or vaccination. They may partially escape immunity and become more contagious. This category includes variants described in Brazil and United Kingdom, known as P.2 and B.1.525 and a variant associated with a rapid spread in New York, B.1.526. The second category is Variants of Concern or variants of public health importance (VOC), which may cause more severe infections with increased hospitalization and high mortality rate as they are more contagious and virulent than VOIs. It includes B.1.1.7 (also known as Alpha) lineage first detected in the United Kingdom, identified as the first major variant of concern which has now been detected in at least 80 countries. Other VOCs include Beta, Gamma, and Delta (Figure 1). The third category that could cause more severe clinical manifestation is known as variants of high consequence, providing anti-viral-resistant variants and variants with real loss of preventive efficacy of vaccines, antibodies, and monoclonal therapy. Till now, none of the SARS-CoV-2 variants has been classified in this category (Henry and Walke, 2021; Lauring and Malani, 2021).

As with all viruses, SARS-COV-2 will continue to evolve if it continues to spread. The more the virus spreads, the more pressure there is for the virus to change. So, the best way to prevent more variants from emerging is to stop the spread of the virus (Halim, 2021). As many people live with innate and acquired immunosuppression, viral evolution in immunocompromised patients can be essential in emerging new variants. Thus, with persistent SARS-CoV-2 infection, rapid viral evolution has been described in immunosuppressed patients, which can be a vital factor in the emergence of such variants (Corey et al., 2021). The emergence of new variants highlights the importance of early identification and genomic surveillance for predicting future variants (Abdool Karim and de Oliveira, 2021).

Mutations in the spike glycoprotein are an area of high interest that potentially compromise vaccine effectiveness by escaping from host antibodies (Lauring and Hodcroft, 2021). The emerging SARS-CoV-2 variants demand effective vaccinations. For more effective vaccines and to control the

spread of SARS-CoV-2, nanotechnology plays a vital role in fighting against SARS-CoV-2 and controlling the transmission rate that will, in return, control new variations. The hope for a quick ending to the pandemic was dampened despite the remarkable efficacy of mRNA vaccines against the original SARS-CoV-2 strain due to the high mutation rate. Many studies indicate that the transmission rate and virulence, along with reduced neutralization, have been increased by new variants, making them even more dangerous and troublesome. The most profound contribution of nanotechnology is the development of successful and highly effective nanotechnology-based messenger RNA vaccines. Nanotechnology offers other solutions, including ACE2-based nano-decoys, engineered neutralizing antibodies, and nanoparticle vaccine-elicited neutralizing antibodies. Mutation in the S protein of SARS-CoV-2 decreases antibody neutralization and increases its transmission. Thus, targeting the inhibiting of ACE2 receptors' interaction with the S protein of the variants through nanotechnology could be the most straightforward and promising strategy. The key advantage of nanoparticle-based vaccines, particularly mRNA-Lipid nanoparticles-based vaccines, includes modularity, rapid manufacturing, and high efficacy (Huang X. et al., 2022).

4 Nano-hygiene; the potential of nanotechnology in preventing variants of concern

Prevention and mitigation against SARS-CoV-2 are as crucial as the other two pillars of diagnosis and therapeutics to combat the virus (Kamat et al., 2021). The first case of SARS-CoV-2 was reported in 2019, which quickly spread like wildfire, with cases in almost every country in no time because of its high transmissibility rate (Petersen et al., 2020). It is transmitted to humans directly or indirectly by air passage and touching contaminated surfaces (Cirrincione et al., 2020). To block its transmission route, it was initially recommended to use personal protective equipment such as masks, gloves, and clothes, use hand sanitizers, and avoid public gatherings that work up to some extent (Cirrincione et al., 2020). Otherwise, it causes a burden on healthcare centers and pharmaceutical industries (Mallhi et al., 2020). To solve these rising problems, researchers are searching for the most suitable solutions; ultimately, nanotechnology is one of the most promising solutions. Nanomaterials can be essential in preventing the spread of SARS-CoV-2 and its variants (Table 1) (Ramakrishnan et al., 2021) because of their distinctive properties, as mentioned before. Nanotechnology aid as a helping hand against SARS-CoV-2 prevention in the following ways.

4.1 Nanomaterials in personal protective equipment

The rate of infection of SARS-CoV-2 is much higher than the previous epidemic SARS-CoV-1. Despite several preventive measures, many people have been infected with SARS-CoV-2. One of the factors, due to which, healthcare workers as well as public became vulnerable was the use of contaminated Personal Protective Equipment (PPE) in hospitals (Sportelli et al., 2020). Nanotechnology based strategies could provide better protection when applied during the manufacturing of PPE. These include the use of angiotensin-converting enzyme-2 (ACE-2) coated nanoparticles; iron oxide coated nanomaterials, and coating by silver nanoparticles, *etc.* Using these nanomaterials in face masks, eye-protecting glasses, hand sanitizers, and clothes can exhibit antiviral effects, and thus a higher prevention capacity to avoid spread and hence the emergence of SARS-CoV-2 variants (Dhama et al., 2021; Mohapatra et al., 2021).

4.1.1 Protective masks and the potential of nanotechnology

Protective masks based on the use of nanomaterials promise a remarkably effective way of preventing spread of SARS-CoV-2. For instance, a type of protective mask based on graphene nanomaterials has been developed by directly transferring graphene layers onto disposable surgical masks *via* a dual-mode laser-induced transferring technique (Pal et al., 2021). It was reported that graphene-coated masks possess self-cleaning abilities. Additionally, due to the hydrophobic nature of laser-modified graphene, it repels the incoming respiratory droplets. Similarly, these laser-modified mask temperatures can attain up to 80°C due to sunlight-induced photothermal activity and thus kill any microorganisms. This means that these masks could be used for a long time because of its self-disinfection and reusability against bacteria and viruses.

Similarly, the laser-induced transfer method has directly fabricated plasmonic silver nanoparticles on N95 masks. It also showed self-cleaning abilities with better protection, releasing silver ions with respiratory droplets against bacteria and the SARS-CoV-2 virus (Kiremitler et al., 2022). Furthermore, Aydemir and Ulusu (2020) reported coating masks and nasal filters with ACE2 enzyme for protection against SARS-CoV-2. The PPE fabrics woven by polyamide 6.6 fibers (PA66) embedded with zinc ions were reported to reduce the Influenza A H1N1 virus and SARS-CoV-2 titer and give protection against the viral spread (Gopal et al., 2021). The advantage of this embedded fabric is that it provides protection even after 50 washes. Similarly, Hewawaduge et al. (2021) reported the inactivation of SARS-CoV-2 by incorporating Copper Sulfide in the three-layer mask to be a lifesaver during previous and future pandemics.

TABLE 1 Different nanomaterials and their effects in the prevention of SARS-CoV-2.

Nanomaterials	Applications	Mechanism of action	References
Graphene nanomaterials	It gives self-disinfection property to mask	Graphene layers are directly transferred through dual-mode laser-induced technique onto disposable surgical masks, which give it self-cleaning ability and resist incoming respiratory droplets	Kiremitler et al. (2022)
Zinc Oxide	Used as a surface disinfectant	ZnO-NPs cause cleavage of oligosaccharides from the glycan shield by hydroxyl radicals and ROS-mediated degradation of viral entry. Thus, due to its high cytotoxicity, it is used as a surface disinfectant	Lin et al. (2021)
Copper sulfate	Used for making the protective mask	Incorporation of copper sulfide in three-layer masks prevents SARS-CoV-2 transmission	Mohapatra et al. (2021)
Nanofiber membrane	Used to make reusable PPE and for air filtration	The zeolite imidazole framework (ZIF) is an effective adsorbent. Using the electrospinning technique, ZIFs are added to the nanofiber to improve the capacity to filter hazardous gases and particulate matter. This new class of reusable filter fiber improves the air permeability	Liao et al. (2021)
Tritium Oxide	Inactivate Sars-CoV-2 in water	TiO_2 inactivates viral propagation due to its photocatalytic mechanism. The electrons are excited that releasing ROS, causing viral inhibition	Magalhaes et al. (2017)
Copper	Inactivate SARS-CoV-2 by fragmenting the viral genome on surfaces	Copper inactivates the virus by the release of copper ions and the generation of ROS	Poggio et al. (2020)
Lipid nanoparticles	Nanovaccines-effective against alpha (B.1.1.7 lineage) and delta (B.1.617 lineage) VOC	The oral vaccines based on self-replicating RNA lipid nanoparticles neutralized both alpha and delta variants of SARS-CoV-2	Mohammadi et al. (2021)
Gold nanoparticles	Nanovaccines-boosted immunity	Using antigen-colloidal gold complex as a vaccine boosted T cell proliferation tenfold more than using only the free antigen, which improved respiratory macrophage performance and boosted protection	Raja et al. (2021)
Zinc oxide nanoparticles	Inactivate SARS-CoV-2 in water	ZnO nanoparticle is commonly used for wastewater disinfection, and in the presence of sunlight, it is reported to be more efficacious	Al-Gheethi et al. (2020)
Silver nanoparticles	Used as a surface disinfectant	Silver is used as antimicrobial, silver atoms bind with thiol (SH) and disulfide (S-S) groups present in bacterial cell membrane proteins, causing membrane disruption and necrobiosis	Ghedini et al. (2021)
Iron oxide nanoparticles	Used to make eye-protecting glasses	Iron oxide nanoparticles coated glasses reduce SARS-CoV-2 viability up to 90%, exhibit antiviral effects, and prevent the spread of the virus	(Dhama et al., 2021; Mohapatra et al., 2021)
ACE2 proteins coated nanoparticles	Used to make gloves	SARS-CoV-2 enters the body through the conversion of ACE2 receptors. Due to this, the ACE2 proteins coated on nanoparticles are used to make gloves helpful in the control of infection	Owida et al. (2022)
Tritium oxide nanoparticles	Used to make protective masks	TiO_2-based photocatalytic materials have prepared nanowire-based filters for the face masks. Due to the photocatalytic capability in the masks, ROS produce upon illumination destroy the virus, causing damage to its proteins, nucleic acid, and lipid membrane	Karmacharya et al. (2021)
Carbon nanotubes	Eliminate the virus from the surface of PPE	Positive surface charges are used over the metallic surface coated by carbon nanotubes using lithography. Then these coated surfaces are fixed on the most exposed part of the PPE. It will destroy the SARS-Cov-2 when it comes in contact with the vicinity of charged surface of healthcare workers	Kashyap and Saha, (2020)
Copper nanoparticles	Used in facemask as a face shield against SARS-CoV-2	Cu has antiviral and antimicrobial activity. It causes viral destruction by ROS formation and destroys viral genetic material. It could be used to make antiviral masks to prevent the SARS-CoV-2 spread	Foffa et al. (2022)

(Continued on following page)

TABLE 1 (*Continued*) Different nanomaterials and their effects in the prevention of SARS-CoV-2.

Nanomaterials	Applications	Mechanism of action	References
Silver nanoparticles	Mouthwash and nose rinse with AgNPs prevent the SARS-Cov-2 infection rate	In the oral and nasal cavity, the viral load is decreased by rinsing it with AgNPs solution and temporarily reducing the risk of transmission	Almanza-Reyes et al. (2021)
Graphene oxide	GO used in textiles, filters and masks inactivate the virus	Due to the hydrophobic nature of GO, microorganisms couldn't penetrate the protective layers of the facemask and are easily recyclable by photocatalysis or heat	Ghaemi et al. (2021)
Ferritin-based nanoparticles	Used in vaccines for prevention against viruses	Ferritin-based nanoparticle assembly mediated by RNA could induce $CD4^+$ T cell activation, which elicits the production of IFN-γ and TNF-α against MERS-CoV	Rasmi et al. (2021)
Metallic nanoparticles	Used to develop antiviral, antimicrobial, and reusable face masks	A face mask was developed by the incorporation of a filtration system composed of a polylactic acid and cellulose acetate nanofibrous matrix containing copper oxide nanoparticles (CuONPs) and graphene oxide nanosheets and produced by electrospinning technique	Chue-Gonçalves et al. (2021)
Nanofibers	It is integrated into masks to ensure high breathability and filtration efficiency	Nanofiber membrane has a high surface area due to which it can easily retain virus particles when used in masks and has a greater breathability rate, which means higher comfort and lowers the fatigue	Pandey et al. (2020)
Spike protein nanoparticles	Commonly used in vaccines as a robust neutralizing immunoglobulin response against SARS-CoV-2	In a viral infection, the spike proteins recognize the cell receptors. It's used in vaccines to prevent reinfection by inducing neutralizing antibodies and preventing the virus from entering human cells	Rasmi et al. (2021)

4.1.2 Nanofabricated gloves

In addition to masks, nanoparticles are used in gloves against SARS-CoV-2. It is known that SARS-CoV-2 enters the body through the conversion of ACE2 receptors; blocking the receptors or lowering the quantities of this enzyme in the body may help to control the infection. For this purpose, the ACE2 proteins coated on nanoparticles have shown remarkable potential and chemical reliability, which can be used to make gloves (Mallakpour et al., 2022). These gloves, similar to protective masks, can protect against infection by killing it in coated films.

4.1.3 Reusable personal protective equipment and the potential of nanotechnology

The problem of contamination in PPEs was coupled with the fact there was a shortage of PPE due to high demand. This led to a shortage of PPE and, as a result, a high infection rate among healthcare workers. The public faced a high infection rate supplies with PPE during the pandemic. On the other side, it also caused a burden on the environment. Daily, more than 450 tons of medical waste have been created since COVID-19 started. Scientists are exploring ways to extend PPE usage time while ensuring sterility. Different methods have been explored, like moist heat, hydrogen peroxide, and ultraviolet germicidal irradiation. In this area, the use of nanoparticles is found to be more efficacious (Xu et al., 2021). However, more effective, and safe strategies were needed that could decontaminate the existing PPE to reduce spread.

The mask has a central layer of zeolitic imidazolate framework (ZIF-67) trapped in a polystyrene hierarchical porous nanofiber membrane (ZIF-67/PS HPNFM), which provides effective filtration of airborne hazardous substances and particulate matter. Because of their high porosity, surface area, and stability in an acidic environment, ZIFs make an effective adsorbent. Using electrospinning technology, ZIFs could be added to the nanofibers to improve their capacity to filter hazardous gas compounds and particulate matter. This new class of reusable filter fibers has improved air permeability. The membrane-based masks could filter out pollutants. As a result, these masks could be cleaned with ethanol and water without losing their ability to filter (Liao et al., 2021). SARS-CoV-2 can be neutralized using a novel antiviral technique called photocatalysis. After light irradiation, photocatalytic materials produce reactive oxygen species (ROS) when oxygen is present. These ROS then destroys the virus, causing damage to its proteins, nucleic acids, and lipid membrane. Titanium oxide (TiO_2)-based photocatalytic materials have prepared nanowire-based filters for facemasks. The photocatalytic capabilities in these masks produce ROS upon UV illumination. To effectively trap pathogens of various sizes, the size of the facemask filter can be adjusted during the manufacturing of TiO_2 nanowires on the filter paper. It has been shown as easily sterilizable, reusable, and

antiviral, thus acting as a powerful prophylactic weapon against rapid transmission of SARS-CoV-2 (Karmacharya et al., 2021).

4.2 Using nanotechnology for surface disinfection

To prevent the transmission of SARS-CoV-2, one approach is to slow down the dissemination of the virus by disinfecting air, skin, or surrounding surfaces. For this purpose, chemicals are used (such as alcohol, peroxides, quaternary amines, and chlorine). Still, they have certain limitations, such as 100% viral inhibition being required in high amounts, ineffective after a short time, and hazardous to public health and the environment. It has been reported that nanostructure coated surfaces reduce SARS-CoV-2 viability up to more than 90% in 10 min to 2 h (Hasan et al., 2020). Consequently, metallic nanoparticles (silver, copper, and TiO_2 nanoparticles) are used as an alternative because of their broad range of antiviral activity, persistence, and effectiveness for a long time at a low dosage (Talebian et al., 2020). These metal nanoparticles are toxic to the pathogen. As discussed earlier, they inhibit microbes directly or through the generation of ROS by disrupting proteins, RNA, intracellular organelles or cell walls, and cell membranes. Among metals, silver is the most used antimicrobial; its bactericidal mechanism is based on the binding of silver atoms with thiol (SH) and disulfide (S-S) groups present in bacterial cell membrane proteins, resulting in membrane disruption and eventual necrobiosis. However, many reports have proved its antiviral effectiveness against several human pathogens such as influenza virus, hepatitis B virus (HBV), norovirus, and SARS-CoV. In a study, AgNPs used in polycotton fabrics inhibited SARS-CoV-2. Several Ag-based sanitizers and disinfectants have also been introduced to sanitize hands and inanimate surfaces (Ghedini et al., 2021). Copper (Cu) has also been considered the best antimicrobial metal. Poggio et al. (2020) studied an antimicrobial potential of a range of copper alloys to inhibit the transmission of Human Coronavirus 229E. The virus inactivation occurs on brass and copper-nickel surfaces at room temperature due to the release of copper ions and the generation of ROS.

Similarly, ZnO-NPs cause cleavage of oligosaccharides from the glycan shield by hydroxyl radicals and ROS-mediated degradation of viral entry. However, due to the high cytotoxicity of ZnO-NPs, it would be best suited to use them as a surface disinfectant to minimize the interaction of the virus with human cells (Lin et al., 2021). Moreover, research has shown to inactivate the SARS-CoV-2 alpha variant (as a VOC) only after 1 min of contact with surfaces by copper-silver (Cu-Ag) nanocomposites. Testing these nanomaterials on three different surfaces showed that Cu (~56, ~59, and ~48 wt%, respectively) is most effective, followed by Ag content (~28, ~13, and ~11 wt%, respectively). These findings suggest that the administration of this nanocomposite as surface disinfectant in highly crowded places (e.g., schools, public transportation, public toilets, hospital, and live-stock reservoirs) potentially halt the spread of the SARS-CoV-2 virus (Mosselhy et al., 2022).

4.3 Inactivation/destruction of SARS-CoV-2 in environmental bodies: Air and water

The pathogenicity and virulence factor of viruses can be determined by their survival rate in the environment and their ability to transmit to humans and animals without a host cell. In healthcare centers, virus containing waste is discarded in the environment after using PPE and other equipment, which can again transmit to humans and animals through air and water. Many treatment strategies are used to inactivate the virus. However, studies have shown that the pathogen fragments remained in effluents. The applications of nanotechnology could prove worthwhile in these cases. The potential of nanotechnology in combination with solar-based disinfection is a novel approach to inactivate human pathogens. ZnO nanoparticles have been used for wastewater disinfection, and they are reported to be more efficacious (Al-Gheethi et al., 2020). TiO_2 nanoparticles possess the potential to decontaminate air filtration systems, aerosols, surfaces, and water by inactivation viral propagation. Due to their photocatalysis mechanism, the electrons are excited from the valence band to the conduction band that initiates the reaction and releases ROS, such as hydroxyl radical and superoxide anion, which gives TiO_2 its disinfectant properties against viruses (Magalhaes et al., 2017). Activated carbon nanomaterials could control the variants of SARS-CoV-2 because of their superior ability to capture viruses. Commercially available powdered activated carbons capture viral particles in their nanopores and eliminate them through hydrophobic interactions with the virus surface to remove them from the environment. These findings indicate a good strategy for the controlled elimination of SARS-CoV-2, which can spread through aqueous media (Ruiz-Hitzky et al., 2020).

Similarly, an efficient nano-enabled photoelectrochemical oxidation is considered an excellent choice to provide virus-free air. This air purifier can trap indoor microorganisms (bacteria and viruses, including coronaviruses), molds, allergens, and other air pollutants. It is observed that after testing this technology, it is found to be more efficient as an air purifier (Kaushik and Dhau, 2022).

4.4 Nano vaccines; the utilization of nanotechnology in the development of vaccines

In achieving an effective response to sudden pandemics of such as COVID-19, significant challenges remain. Although, the

considerable success of delivering conventional viral vaccines could generate solid antiviral responses. Due to concerns about traditional vaccines, like weak immunogenicity, toxicity, intrinsic instability *in vivo*, and the need for multiple administrations, improvements and further progress are required (Kim et al., 2014; Huang et al., 2021). The use of nanotechnology in the production of vaccines gives another solution to these problems (Abd Elkodous et al., 2021a). Nanoparticle-based antigen delivery has many advantages over traditional vaccine delivery systems, such as safe delivery vehicles, vaccine adjuvant, improved antigen stability, targeted delivery, long-time controlled release, and evasion of immune responses. Nanotechnology-based vaccines are easier to design, synthesize, and scale up in a larger volume than traditional vaccine approaches (Malabadi et al., 2021).

To enhance the humoral and cellular immune responses, nanocarrier-based delivery systems provide opportunities to overcome such problems. Numerous vaccine nanocarriers have been designed and investigated to promote a protective immune response for the utility of antigens and adjuvants to immune cells. For proper vaccine delivery *via* oral or mucosal routes, solid nanocarriers can facilitate entry into the gut-associated lymphoid tissue and mucosa-associated lymphoid tissues and protect protein-based antigen vaccines from degradation (Kim et al., 2014). Nanoparticles have been developed with diverse functions by taking advantage of pathogen-adopted specific delivery and translocation mechanisms known as bioinspired or biomimetic nanoparticles with good biocompatibility, extended circulation time, and enhanced accumulation at the infection sites. To design more effective vaccine formulations, biomimetic nanoparticles have unique immunostimulatory properties and antigenic characteristics. MERS-CoV-specific IgA cannot persist for an extended period while protecting patients with the infection. The delivery of biomimetic nanoparticles protects the adjuvant from degradation. Also, it protects the body from systemic toxicity caused by free adjuvants, which could cause side effects such as nausea, fever, drowsiness, and diarrhea (Huang et al., 2021). Stable, effective, and target-specific delivery of an antigen can be generated through multifunctional nano vaccines that can significantly increase the immune response. However, production costs and manufacturing processes can be improved for many-component nano-vaccines with complex structures. To enhance the immunogenicity of a vaccine antigen, nanotechnology-based vaccine delivery systems have been developed by modulating antigen delivery to the immune cells (Borges et al., 2005).

Working mechanisms of nanotechnology-based vaccine formulations have supported the utility of nanocarriers in vaccination. Phagocytic cells, such as macrophages and dendritic cells (DC), readily take up particles smaller than 10 μm. The efficiency of antigen recognition and presentation has improved the cellular uptake of antigens through this property (Oyewumi et al., 2010). To eradicate SARS-CoV-2 completely and thus reduce the risk of emergence of VOCs, immediate control measures in terms of vaccination are essential. This crisis also demands an urgent analysis of all the available nanotechnology tools. For designing the vaccine carrier, nanomedicine strategies are used. Since nanomaterials are ideal for antigen delivery as adjuvants, nanotechnology benefits the modern vaccine design and mimics the structural features of viruses. In the smooth delivery of the antigen into the host cell, nanoparticles help trigger antigen-specific immune responses. Antigens can either be encapsulated inside the nanocarriers or bound (conjugated) to the surface of the nanoparticles and administered together with the adjuvant to the target (Malabadi et al., 2021).

The key target for vaccine development, therapeutic antibody generation, and the clinical diagnosis of COVID-19 is spike S protein (glycoprotein). S protein helps the virus to enter the target cells. Still, this endocytosis simultaneously depends on the initiative activation of S protein by cellular proteases and the binding of S protein to membrane ACE2 receptors. Therefore, a vaccine against the S protein prevents the proliferation and spread of SARS-CoV-2 (Huang X. et al., 2022). In current clinical trials for SARS-CoV-2, nanotechnology-based vaccines are the leading alternative to viral vector vaccines. The recent support of two lipid nanoparticle-based vaccines has demonstrated that nanomedicine can be an important asset in controlling VOCs. The delivery of drugs and molecules is facilitated by nanomedicine that would otherwise be useful or viable therapeutics (Milane and Amiji, 2021).

The prefusion spike protein (Pre) of SARS-CoV-2 used in vaccines neutralizes SARS-CoV-2 variants but elicits less antibody-dependent cellular cytotoxicity activity than S2 proteins. S2 proteins induce IgG antibodies with potent and broad antibody-dependent cellular cytotoxicity (ADCC) activity but weaker neutralization. The immunogenicity of S2 and Pre has been shown to improve by incorporating them into double-layered protein nanoparticles. The resulting protein nanoparticles Pre/S2 elicit higher neutralizing antibodies than Pre alone and stronger ADCC than S2 alone (Ma et al., 2022).

Viruses primarily enter our bodies through respiratory droplets. To stop its transmission, it is essential to stimulate mucosal immunity. Inhalable bionic virus nano-vaccines have been used to raise respiratory mucosal immunity to prevent the virus. This nano-vaccine structure is like SARS-CoV-2, consisting of nucleic acid, capsid, and spike protein. When administered through the nasal route, it activates cytokines and helps the receptor binding domain to mature helper T cells and B cells (Zheng et al., 2021). Nasally administered hACE2 mice had higher mucosal immunity response capacity and produced high titer sIgA against SARS-CoV-2 in the respiratory mucosa compared to mice that received the vaccine intramuscularly and intraperitoneally. The outcomes of the virus challenge experiment showed that the inhalable nano-vaccine approach could effectively protect the body (Huang J. et al., 2022).

Similarly, the efficacy of oral vaccines based on self-replicating RNA lipid nanoparticles has been tested against

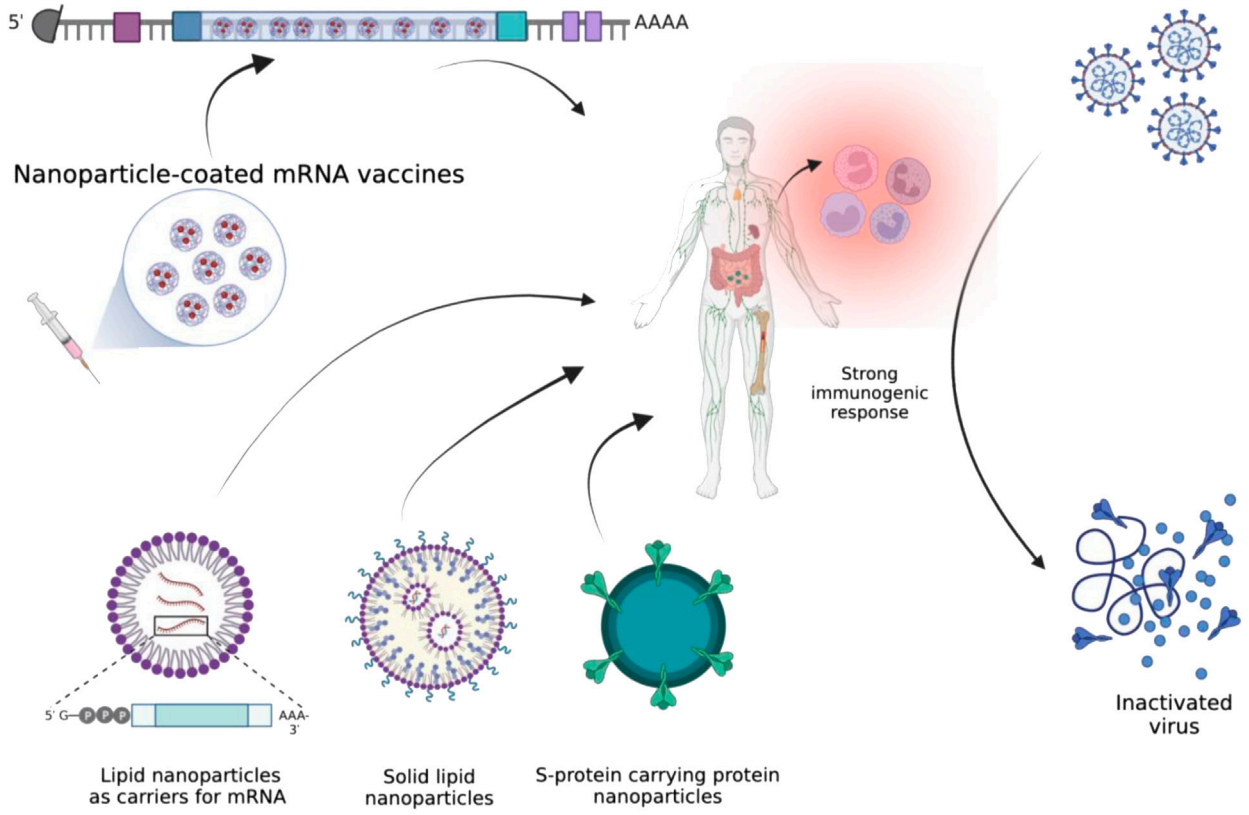

FIGURE 2
Illustration of different types of nanomaterials that could help in eliciting a strong immune response against SARS-CoV-2.

SARS-CoV-2 variants (alpha and delta, i.e., B.1.1.7 and B.1.617, respectively). The study reported that a high quantity of SARS-CoV-2 specific IgG and IgA antibodies were observed after administering RNA LNPs-based vaccines (Figure 2). It is reported to simultaneously neutralize the alpha and delta variants of SARS-CoV-2 (Mohammadi et al., 2021).

Although vaccines against SARS-CoV-2 have been successfully established, the emerging VOCs are still a challenge in their complete prevention. Nanotechnology applications for developing vaccines are a way forward with efficient vaccines. As discussed, the application of nanomaterials to the development of vaccines could cause the direct and indirect spread of the disease and, thus, the emergence of VOCs (Kamat et al., 2021; Mohamed et al., 2022).

5 Diagnostic value of nanotechnology for controlling SARS-CoV-2 variants

Diagnostics proved critical in containing COVID-19 and allowed rapid deployment of control measures to prevent virus transmission by tracing and isolating people (Chau et al., 2020). Researchers have always aimed to develop highly sensitive and effective techniques for identifying pathogens (Table 2). Nano-sized materials can initiate highly effective surface contacts between the analyte and the sensor used for detection resulting in rapid, reliable, and accurate virus detection. Nanomaterials are also beneficial for the bioconjugation of molecules, plus high surface energy. Nanotechnology is expected to improve cellular and subcellular diagnostics using high-throughput sensors and imaging techniques (Kamat et al., 2021).

5.1 Nanotechnology-based biosensors

Biosensors have been created to detect human immunodeficiency virus, influenza, and other viral infections (Misra et al., 2022). Initially constrained by poor sensitivity and specificity, nanotechnology significantly influences biosensors because the search to produce a portable, quick, low-cost, and multiplex device is crucial. Nanotechnology makes detecting many analytes easier,

TABLE 2 Different types of nanomaterials and their diagnostic applications in detecting SARS-CoV-2.

Mechanism of diagnostic tool	Type of nanoparticles	Target	Test duration	Limit of detection	References
Opt magnetic biosensor	IONPs	RdRp	1 h 40 min	0.4 fM	Tian et al. (2020)
Localized surface plasmon coupled fluorescence (LSPCF) probe	Gold nanoparticles	Nucleocapsid protein serum		1 pg/ml	Huang et al. (2009)
Lateral flow immunoassay	Gold nanoparticles	IgM and IgG	15MIN	N. A	Li et al. (2020)
Colorimetric assay	Gold nanoparticles	RdRp	30 min	0.5 ng	Kumar et al. (2020)
Electrochemical immunosensor	Gold nanoparticles	Antigen	-	90 fM	Mahari et al. (2020)
Fluorescence-linked immunoassay	QDs and $MnFe_3O_4$ Nanospheres	IgG	-	4 pg/m	Guo et al. (2020)
Lateral flow immunoassay	SiO2@Au@QD nanobeads	IgM and IgG	15 min	N. A	Rabiee et al. (2022)
plasmonic photothermal (PPT) effect and localized surface plasmon resonance-based biosensor	Gold nanoparticles	RNA	-	0.22 p.m.	Qiu et al. (2020)
Field-effect transistor biosensor	Graphene Oxide nanosheets	S protein	-	2.42×10^2 copies/mL	Seo et al. (2020)
Piezoelectric immunosensor	Piezoelectric crystal consists of quartz wafer	Antigen (sputum)	-	0.6 μg/ml	Zuo et al. (2004)
Lateral flow assay	SA-DNPs	ORF1ab and N	2 min	12 copies/25 μl	Zhu et al. (2020b)
FET	Graphene sheets	Antigen	-	1.6×10^1 pfu/ml	Seo et al. (2020)
Colorimetric assay	Gold nanoparticles	RNA	10 min	0.18ng/μl	Moitra et al. (2020)
Fluorescence-linked immunosorbent assay	IgG-coupled QDs	IgM and IgG	15 min	4 pg/ml	Derakhshan et al. (2021)
Lateral flow immunoassay	Selenium nanoparticles	IgM and IgG	10 min	N/A	Bayin et al. (2021)
Optical immunosensor	Quantum dots	Antigen (nucleocapsid protein)	-	0.1 pg/ml	Roh et al. (2011)

mass transferring information across short distances and tailoring sensing of a wide range of analytes for fast action (Pradhan et al., 2021). Moutaouakil et al. (2020) hypothesized that the integration of nanomaterials into biosensors would improve rapid detection, specificity, sensitivity, integration of nanoscale measurements, and new label-free detection techniques due to their unique optical, physical, mechanical, and electrical properties. Nano-based biosensors have the advantage of biocompatibility and compactness. The fascinating optical properties of nanoparticles, such as surface plasmon resonance, are used in diagnostics to enhance electromagnetic radiation phenomena (absorption and scattering). Similarly, quenching, and fluorescence features of graphene, silver, gold, and other metal oxides-based nanomaterials are features commonly used in biosensors (Yildiz et al., 2021).

For instance, graphene-based biosensors are ideal for various applications due to their high electrical conductivity and surface area. They can be used to detect the coronavirus S antibody (Özmen et al., 2021). Similarly, when AuNPs and AgNPs linked to antibodies attach to RNA or viral antigens, a detectable signal can identify SARS-CoV-2 (Maddali et al., 2021). Furthermore, toroidal plasmonic meta-sensors that detect viral protein S concentrations in the femtomolar range have been produced. Monoclonal antibodies conjugated to functionalized AuNPs have been seen at dosages of up to 4.2 fmol (Ahmadivand et al., 2021).

Researchers have recently developed a one-step Protein-S-specific nano-plasmonic resonance optical sensor that detects viruses quickly and directly with little sample preparation. Antibodies highly specific against SARS-CoV-2 have been restrained on surfaces of nano-sensor chips to which whole coronavirus particles adhere *via* protein S, resulting in

plasmon resonance or intensity changes that can be monitored optically with a detection device (Rhouati et al., 2021). Nano-plasmonic sensor chips offer the advantage of being inexpensive and scalable while maintaining consistency and repeatability. The flexible and non-contactable nature of the nanostructures allowed the researchers to integrate the sensors into a standard 96 microplate or a microfluidic cuvette, which enables them to be used for measurements (Iravani, 2020).

Similarly, a wearable device using AuNP-based sensors combined with artificial intelligence that can be controlled using a smartphone app can detect SARS-CoV-2 in just 15 min in exhaled breaths. This detection identifies viruses based on variations in the resistivity of the nanomaterial biosensor layer (Torrente-Rodríguez et al., 2020). Plasmonic detection technology can significantly reduce the frequency of false positives. A clinical diagnostic test was created by the researchers having a dual function like a surface plasmon resonance sensing transduction and plasmonic photothermal. The experiments take place on two-dimensional gold nano-islands. SARS-CoV-2 nucleic acids hybridize to complementary DNA receptors on gold Nano islands. This device can be stimulated with different wavelengths because of two separate angles of incidence: one from localized surface Plasmon resonance and the other from a plasmonic photothermal biosensor. It can recognize the envelope genes (E) of RdRp-COVID, F1ab-COVID, and SARS-CoV-2. The dual-purpose localized surface Plasmon resonance biosensor can identify specific SARS-CoV-2 sequences in a multigene mix and has a detection limit of 0.22 p.m. (Qiu et al., 2020).

Researchers have also created a plasmonic nano-hole array that transmits light for label-free pathogen detection in biological conditions without sample preparation. Capturing intact virions on immobilized group-specific antiviral immunoglobulins on the sensor surface allows you to quantify them. The virus attaches to a suspended Nano hole network that connects incident light to surface Plasmon's, causing the surface Plasmon resonance frequency to shift red (Kevadiya et al., 2021). Silicon nanowires are being developed as ultra-sensitive mark-free sensor technology. After being modified with antibodies, silicon nanowires transistors can detect a single virus (Preetam et al., 2022).

Layqah and Eissa (2019) developed an immune sensor using AuNPs coated with carbon electrodes. In this method, the spike protein of COVID-19 acts as an interactive bridge between the virus and the small portion of antibodies present in the sample. At the same time, the actual differences are used for virus identification. If the presence of the virus is negative in the sample, the binding of the antibody to the SI protein induces the generation of a very low current spike. If the presence of the virus is positive, a high current spike is generated. However, the detection limit ranges between 0.4 and 1.0 pg/ml. Ishikawa et al. (2009) employed nanowires instead of nanoparticles to detect SARS-CoV-2.

Chiral biosensors have been suggested as a vital tool for diagnosing SARS COVID-19 virus. A study reveals that the COVID-19 virus was revealed by opening chiral zirconium quantum dots. When the virus is present, magnetic nanoparticles and zirconium quantum dots interact, resulting in magneto plasmonic light. The virus was identified by measuring the fluorescence intensity of fluorescent magneto Plasmon monohybrids separated by external magnets (Asdaq et al., 2021). Another study employed chiral immune sensors made of self-assembled layers of quantum dots and chiroplasmic AuNPs. COVID-19 can be identified in blood samples by mixing quantum dots and AuNPs with the viral sample; modifications of the chiral optical response are disclosed using the circular dichroism technique, which has a low detection limit (47.91 EID/50 L) (Ahmed et al., 2017). Overall, biosensors and other materials science-based detection techniques have the potential to provide rapid and portable SARS-CoV-2 diagnostic tests.

5.2 Immunochromatographic test

Diagnostic tests for coronaviruses have used metallic, polymeric, silica, iron oxide, and quantum dot nanoparticles. Among these, AuNPs are by far the most commonly used metal systems. These nanoparticles are broadly used because their surface plasmons have the characteristic wavelength-selective absorption with relatively high molar extinction coefficients. An AuNP-based colorimetric assay was developed and complemented with thiol-modified antisense oligonucleotides with sufficient specificity for the SARS-CoV-2 N-gene. As a result, it demonstrated the possibility of selective and rapid (10 min) diagnosis of COVID-19 with the naked eye (Kevadiya et al., 2021). When the SARS-CoV-2 target RNA sequence was present, thiol-modified ASOs covered the AuNPs aggregate, and a change in surface plasmon resonance was observed. However, the amount of virus injected may affect the test (Moitra et al., 2020). As in a colorimetric assay, SARS-CoV protein E was immobilized on AuNPs using an Au-binding polypeptide that plays a role of a mediator. These AuNPs were also functionalized using a GFP, and when they interacted with a complementary protein antibody, they changed their color and absorbance, allowing measurable detection of SARS-CoV (Rasmi et al., 2021). AuNPs with a diameter of 17 nm have also been used in a biosensor to detect SARS-CoV based on fluctuations in fluorescence (Gao et al., 2021). The colloidal gold immunochromatography test is a simple and rapid screening method widely used in various disciplines to diagnose diseases. It has several advantages, including speed, affordability, and simplicity. Serologic point-of-care (POC) diagnostics are based on lateral flow immunoassay (LFIA) methods (Figure 3). The FDA has approved many LFIA-based POC diagnostics (Wang et al., 2021a). One of these tests is Cellex Inc.'s COVID-19 IgM/

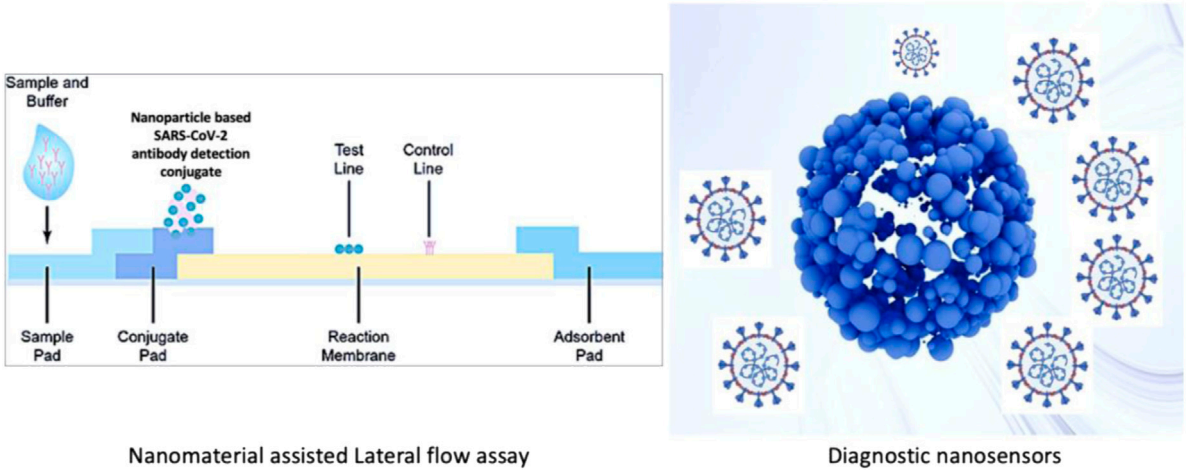

FIGURE 3
Images demonstrating the diagnostic value of nanotechnology in detecting SARS-CoV-2.

IgG rapid test, which allows for the identification and qualitative differentiation of IgM and IgG antibodies against SARS-CoV-2 in 15–20 min (Mahmoudinobar et al., 2021).

5.3 Polymerase chain reaction coupled with nanosensors

The nucleic acid of SARS-CoV-2 is detected using real-time RT-PCR. However, the RT-PCR diagnostic kit provides a wide range of false positive or negative results. Nanomaterial-based technology offers viable alternatives to RT-PCR for rapid and accurate virus detection (Engelmann et al., 2021). For instance, 3 aminopropyl triethoxysilane and coprecipitation of magnetic nanoparticles can be employed to simplify the extraction of viral RNA and the functionalization of polyamine ester, which could then be used in as many as 50,000 diagnostic tests (Chacón-Torres et al., 2020).

To identify the genetic sequences of SARS-CoV, silica-coated superparamagnetic nanoparticles were used at different stages of polymerase chain reaction (PCR) experiments (Gong et al., 2008). A recent work uses polystyrene nanoparticles doped with lanthanides that produce fluorescence capable of detecting the presence of viruses, the results obtained by RT-PCR (Maddali et al., 2021).

Chacón-Torres et al. (2020) examined magnetic nanoparticles by isolating SARS-CoV-2 virus RNA using RT-PCR techniques. These nanoparticles employ polymers to capture RNA on their surfaces through carboxyl groups (amino ester). The carboxyl group is employed in a combination of RNA magnetic nanoparticles for RT-PCR diagnostics. This process takes lesser time and is relatively safe. In another study, magnetic nanoparticles were used to isolate virus particles rather than extraction. The findings show that virus particles bind tightly to nanoparticles through functionalized targeting receptors. Superparamagnetic iron oxide nanoparticles were used because their external magnet helps separate virus particles (Vahedifard and Chakravarthy, 2021). Zhu et al. (2020a) used one-step reverse transcription loop-mediated isothermal amplification conjugated with nanoparticle-based biosensors in a single-tube reaction to diagnose COVID-19 accurately and quickly. The diagnostic system produced has a sensitivity of 12 copies per reaction and no cross-reactivity with templates other than COVID-19. In oropharyngeal swab specimens from COVID-19 patients, the developed RT-LAMP-NBS has a sensitivity and specificity of 100%. Conclusively, nanomaterials used in PCR experiments reduce reaction time, increase signal amplification selectivity, and improve detection sensitivity.

6 Treatment strategies

When a new viral disease emerges, no vaccine is available for it immediately, and people have to rely on available antiviral therapeutics, which are effective initially but can also cause adverse effects that outweigh the benefits (Zhou et al., 2021). Since the epidemics of coronaviruses such as SARS-CoV and MERS-CoV, two decades have passed, but still, no efficient drug against coronavirus has been approved so far. For this purpose, nanotechnology opens new avenues that have converged enormous attention and have already been studied for its potential applications in treating viral infections, including COVID-19 (Table 3) (Campos et al., 2020; Vahedifard and

TABLE 3 The types of nanomaterials applied in different treatment strategies against SARS-CoV-2.

Nanomaterials	Function	Potential role against SARS-CoV-2	References
Polymeric nanoparticles	Easily penetrate and absorb in the cells and allow the continuous release of antivirals to the target site after days or even weeks of administration	Colloidal antiviral drug carrier	Khalil et al. (2011)
Chitosan nanopolymer	Directly deliver drugs to the site of infection	Pulmonary delivery of saquinavir to the infected lungs of COVID patient	Chowdhury et al. (2021)
Poly Lactic-co-Glycolic Acid	Nanocarrier for target delivery of drugs	Direct delivery of lopinavir to the site of inflammation; alleviate inflammation	Tan et al. (2021)
Lipid nanoparticles	Encapsulate antiviral drugs for safe target delivery; protect DNA and RNA vaccines from nucleases	Aerosol for the pulmonary delivery of remdisivir; delivery of mRNA and DNA plasmid vaccines	(Mufamadi, 2020; Abd Elkodous et al., 2021b; Tian et al., 2021)
Carbon nanotubes	Increases the uptake efficiency of the drug; blocks the viral entry and inhibits replication	Conjugate with remdisivir and ensure its target delivery; interacts with S protein, ACE2, RdRNP, main protease, and papain-like protease to prevent viral entry and replication	(Neghab et al., 2020; Skariyachan et al., 2021)
Zinc oxide nanoparticles	Inhibit viral replication	Inhibit the synthesis of RdRNP and hence can inactivate replication of SARS-CoV-2	Sarkar and Das Mukhopadhyay, (2021)
Graphene oxide	Alters virus receptor binding proteins and target receptors	Interaction with S protein and ACE2 receptor to block the entry of virus; inhibit viral replication	Unal et al. (2021)
Silver nanoparticles	Disruption of viral integrity; production of ROS; prevention of viral RNA synthesis; block viral attachment to the receptor; interact with receptor binding domain; inhibit viral replication	Potential to be used as anti-SARS-CoV-2 medication	(Das et al., 2020; Jeremiah et al., 2020; Vahedifard and Chakravarthy, 2021)
Iron oxide nanoparticles	Interact with receptor binding domain	Bind with and disrupt S1 receptor binding domain	Jeremiah et al. (2020)
Carbon quantum dots	Block the viral entry	Can prevent the entry of SARS-CoV-2 into the host cell by interacting with the S1 protein	Vahedifard and Chakravarthy, (2021)
Silicon nanoparticles	Interact with several ligands to stop the entry of the virus and also inhibit its replication	Inhibit viral replication	Asdaq et al. (2021)
Carbon nano fullerenes	Block all the targets required for viral entry and replication	Interact with the major structural targets of SARS-CoV-2 like RdRNP, main protease, papain-like protease, and nucleocapsid protein containing RNA binding domain	Skariyachan et al. (2021)
Poly Lactic-co-Glycolic Acid PEG-DX600	Inhibitor of human ACE2	Able to reach and inhibit targeted ACE2 receptors, thus blocking virus entry	Neghab et al. (2020)
Poly-lactic acid nanoparticles	Carrying vehicle of antiviral drugs	Targeted and safe delivery of chloroquine	Vahedifard and Chakravarthy, (2021)
Mosaic RBD-mi3 nanoparticles	Delivery of heterologous antigens	Arise antibody mediated response to several viral proteins for its neutralization	Cohen et al. (2021)
Polymeric nanoparticles	Inhibit the initiation of infection	Attach to ACE2 receptor, inhibit the cleavage of angiotensin	Vahedifard and Chakravarthy, (2021)
Nitric Oxide nanoparticles	Produce peroxynitrite that causes cytotoxic effect and inhibits viral replication	Counteract the SARS-CoV-2 attack on endothelial cells	Cavalcanti and Cajuba de Britto Lira Nogueira, (2020)
Exosomes	Bind to the target and prevent the cellular uptake of the virus, and inhibit replication	Can block the entry and replication of SARS-CoV-2	Hassanpour et al. (2020)
Gold nanoparticles	Deform virus, collapse viral capsid	Have the potential to disrupt the integrity of SARS-CoV-2 and prevent membrane fusion	Asdaq et al. (2021)
Dendrimers	Encapsulate drugs, strongly interact with the virus	It can be used in the treatment of COVID-19	Asdaq et al. (2021)
Glycyrrhizic acid nanoparticles	Inhibit the proliferation of virus; relieve inflammation	Target lungs and alleviate inflammation and reduce viral load	Zhao et al. (2021)

(Continued on following page)

TABLE 3 (*Continued*) The types of nanomaterials applied in different treatment strategies against SARS-CoV-2.

Nanomaterials	Function	Potential role against SARS-CoV-2	References
Titanium oxide nanoparticles	Interact with viral capsid and surface proteins; inhibit replication	It can inactivate the virus by degrading the protein envelope	Shukla et al. (2021)
Small interfering RNA-Lipid nanoparticles	Specifically and directly inhibit viral genome	Adjunctive therapy to vaccines	Idris et al. (2021)
Self-Assembling Proteins based Nanoparticles	Provokes antibody-mediated immunity	They can be applied as nano vaccines against SARS-CoV-2	Rashidzadeh et al. (2021)

Chakravarthy, 2021). This section focuses on the potential of nano-based treatment strategies to fight several viral infections, emphasizing SARS-CoV-2-associated COVID-19 and its emerging variants.

6.1 Nano-carriers for the delivery of antiviral agents

Many antiviral drugs have been developed to treat various viral infections. Though they possess good antiviral activity, several challenges limit their efficacy, including the emergence of drug-resistant viruses, poor solubility, instability during storage and application, low bioavailability, and potential adverse side effects (Delshadi et al., 2021). Nanomaterials can play an essential role in nano-medicine due to their size, shape, and surface charge, which enable them to efficiently deliver therapeutic agents such as drugs (precisely at the proper target and time) and vaccines. Their surface functionalization and size allow them to have antigens and adjuvants for immunization (Gurunathan et al., 2020). The nano-delivery system solves the problems associated with traditional drugs because it enhances the bioavailability, reduces toxicity, protects the drug from *in vivo* degradation, extends circulation time, increases the drug's half-life, and helps it cross biological barriers. The known nano-carriers for antiviral drug delivery are polymeric nanoparticles, metallic nanoparticles, liposomes, dendrimers, micelles, and nanocrystals (Govender et al., 2008; Jindal and Gopinath, 2020).

Polymeric nanoparticles are considered efficient drug carriers because of their high surface area and versatile conjugation properties (Charelli et al., 2022). The biodegradability and small size of polymeric nanoparticles allow them to be used as colloidal drug carriers because they can easily penetrate and absorb the cells and enable the continuous release of the drug to the target site after days or even weeks of administration (Khalil et al., 2011). Chitosan is a natural biocompatible polysaccharide often utilized as a nano-carrier of antiviral medications. Ramana et al. (2014) employed chitosan nanoparticles to load the antiretroviral saquinavir (the most effective medicine for AIDS). They found a drug loading efficiency of 75% and target specificity of more than 92%. It can release antiviral drugs through swelling, diffusion, and erosion mechanisms. The chitosan nanoparticles take advantage of a large surface area in pulmonary drug delivery and directly deliver drugs to the infected lungs of the patient (Chowdhury et al., 2021). In a recent study for the treatment of COVID-19, Tan et al. (2021) developed a nano-carrier drug delivery system by loading lopinavir in PLGA (poly lactic-co-glycolic acid). They then coated the nanoparticles with macrophage membrane to form drug-loaded macrophage biomimetic nanocarriers (PLGA-LPV@M). This nano-carrier relieved inflammation by specifically targeting inflammation sites and neutralizing pro-inflammatory cytokines, macrophages, and neutrophils.

It also reduced viral load (Tan et al., 2021). To treat COVID-19, administering antiviral drugs directly to the lungs is required as it is the primary site of infection, and most deaths are associated with malfunctioning lungs. Li et al. formed liposomal aerosol for the pulmonary delivery of remdesivir to enhance its *in vivo* activity. The encapsulation of remdesivir in liposomes showed high solubility and improved biocompatibility, thus indicating it to be a better option to enhance *in vivo* therapeutic effects for COVID-19 and similar respiratory infections (Peraman et al., 2021). Various *in vitro* and *in vivo* studies revealed the increased bioavailability of drugs in the form of liposomal nano-formulations across the barrier of the mucosal membrane. FDA-approved drugs like doxorubicin and amphotericin B are available in this form (Higgins et al., 2020).

Metallic nanoparticles like silver (Ag), gold (Au), titanium (Ti), zinc (Zn), palladium (Pd), and copper (Cu) have been widely used as nanocarriers for therapeutic agents such as antibodies, drugs, genes, peptides, *etc.* Due to the attractive intermolecular forces between nanoparticles and target drug molecules, the surface of metallic nanoparticles can be easily functionalized to be utilized as an effective carrier of therapeutic drugs (Chandrakala et al., 2022). Metal nanoparticles synthesized from plant extract are desirable for their application in drug delivery because of their reduced toxicity, scalability, and cost-effectiveness (Mehta et al., 2020). Quantum dots (QDs) are nanocrystals with a high binding affinity with therapeutic molecules and target specificity. Some studies showed the target-specific delivery of saquinavir when conjugated with QDs and target DNA, making it an efficient antiretroviral therapeutic agent (Mukherjee et al., 2020). Single-walled

carbon nanotubes (CNTs) have been tested for their drug-carrying capability in fish models. CNTs functionalized with ribavirin showed increased drug uptake and reduced significant therapeutic dosages. Hence CNTs can be employed as nanocarriers for targeted drug delivery (Neghab et al., 2020). Small interfering RNAs (siRNAs) are broad-spectrum antiviral agents that can be designed to degrade the target viral genome. Encapsulation of siRNA in FDA-approved nanomaterials like lipid nanoparticles (LNPs), nano polymers, or lipid-polymer hybrid can be used for the stable and target delivery of siRNA for the degradation of the SARS-CoV-2 viral genome (Ullah et al., 2020).

The development of a novel drug delivery system has envisioned researchers repurposing the drugs (during the unavailability of specific therapeutic agents) effectively for the current and future outbreaks. The developed nano designs for drug delivery can be active against various viral diseases crossing the barriers of patients' heterogeneity. Polymeric, lipid-based, and inorganic nanoparticles are designed in specific ways to carry antiviral drugs in a personalized manner, which can potentially be applied to the population to treat existing and possibly emerging viral diseases (Mitchell et al., 2021).

6.2 Nanomaterials blocking viral entrance into the cell

After reaching the target site, the first step of SARS-CoV-2 is the attachment and entry into the host cell. This attachment and access are facilitated by viral spike glycoproteins targeting the ACE2 receptor in the lungs, heart, kidneys, testis, and intestine cells. Blocking the entry of the virus in the first place is the ideal treatment strategy that can be obtained by applying nanomaterials. The size and surface charge of nanomaterials enable them to attach to the spike protein preventing its binding to the target receptor, thus blocking the initiation of infection (Carvalho and Conte-Junior, 2021). Nanoparticles can directly control the entry of viruses just like AgNPs can inhibit the entrance of respiratory viruses; graphene can hinder the attachment of HIV to the host cell; AgNPs conjugated with graphene oxide (GO) have shown effective activity against feline coronavirus and other enveloped viruses (Chintagunta et al., 2021). Nanomaterials like AuNPs and carbon quantum dots, due to their large surface area to volume ratio, can interact with many biomolecules, including the spike protein of SARS-CoV-2 preventing its entry into the cells (Figure 4). AuNPs mimic a binding receptor of viruses like herpes simplex virus, lentivirus, and human papillomavirus resulting in viral degradation. A study revealed that GO could inactivate viruses before entering the cell. The spike glycoproteins of the viral envelope were destroyed when incubated with GO for 1 h (Vahedifard and Chakravarthy, 2021). AgNPs are also evaluated for their antiviral activity to inhibit SARS-CoV-2. Luciferase-based pseudovirus entry assay showed that 10 nm sized AgNPs in the concentration of 1–10 ppm blocked the entry of this virus by disrupting its integrity (Jeremiah et al., 2020). A docking study revealed the binding of iron oxide nanoparticles with the S1 receptor binding domain, preventing the adsorption of SARS-CoV-2 to the host cell (Chue-Gonçalves et al., 2021).

In addition to the interaction with spike protein, the entry of SARS-CoV-2 into the host cell can be blocked by inhibiting the ACE2 receptor. The studies of human and mouse cell lines showed that DX600 is a specific and effective human ACE2 inhibitor. DX600, in conjugation with nano-copolymer like polylactic-co-glycolic acid (PLGA) and polyethylene glycol (PEG), would be able to reach and inhibit targeted ACE2 receptors (Neghab et al., 2020). The interaction of GO with spike protein, ACE2, and their complex has been studied in a molecular docking analysis which showed the high affinity of GO for both receptor and viral protein. This study revealed the capacity of GO sheets to block infection by disrupting viral spike proteins even with any mutations in them (Unal et al., 2021).

6.3 Nanomaterials inhibiting viral replication

Once SARS-CoV-2 is attached and entered into the host cell, it releases the RNA genome into the cytoplasm, translating it into proteins in viral replication (Harrison et al., 2020). To stop the virus's progress from spreading infection, inhibiting viral genome replication is one of the most successful steps. For this purpose, nanoparticles are desirable antiviral agents that can be designed to help them attach and penetrate the target cell and either directly or indirectly inactivate the virus by blocking its replication (Pilaquinga et al., 2021). The metal and mineral nanoparticles can block various structural targets of SARS-CoV-2 to prevent its replication (Sarkar and Das Mukhopadhyay, 2021). AgNPs have been studied as antiviral agents against many life-threatening viruses and have been revealed to be efficient therapeutic agents having the potential to treat other diseases. AgNPs have shown the inhibition of HIV1 by binding to gp120, preventing the virus's entry and replication by blocking the formation of a complex between virion and CD4. Also, AgNPs have been discovered by Lu and coworkers to stop the production of intracellular HBV RNA. Due to the successful results, AgNPs are suggested by many studies to be employed for the treatment of COVID-19 (Siadati et al., 2020). The antiviral mechanism of AgNPs involves the production of reactive oxygen species (ROS), leading to oxidative stress that damage the viral genome and proteins, initiate an immunological response, and prevent the viral RNA synthesis by blocking the attachment of the virus to its receptor (Das et al., 2020). In a recent study, AgNPs (10 nm) coated with polyvinylpyrrolidone applied *in vitro* to Vero cells infected with SARS-CoV-2 showed almost complete inhibition of viral replication with low

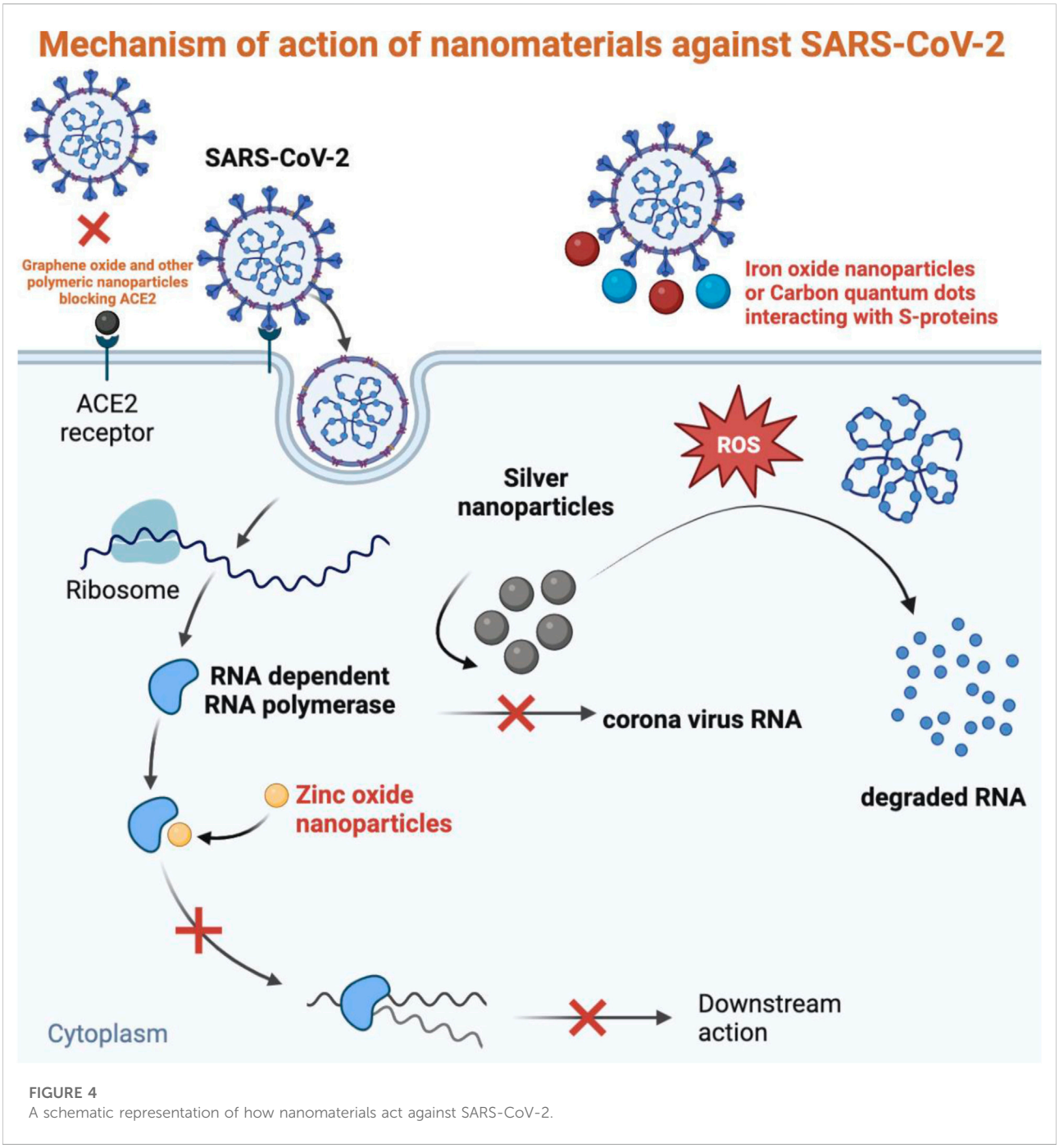

FIGURE 4
A schematic representation of how nanomaterials act against SARS-CoV-2.

toxicity (Jeremiah et al., 2020). Mesoporous silica nanoparticles (SiNPs) can interact with several ligands to stop the virus's entry and inhibit its replication (Asdaq et al., 2021).

Zinc ionophores can block the replication of the viral genome by inhibiting RNA-dependent RNA polymerase (RdRNP) by releasing higher concentrations of Zn^{2+} ions. Metal nanoparticles can produce an increased level of ROS that can penetrate the cell membrane and interfere with RNA and protein synthesis, thus hindering post-attachment viral replication (Sarkar and Das Mukhopadhyay, 2021). A recent molecular docking study has been performed to determine the interaction of CNTs and fullerenes with the significant structural targets of SARS-CoV-2 like RdRNP, main protease, papain-like protease, and nucleocapsid protein containing RNA binding domain. All these proteins play an essential role in the transcription and replication of the viral genome. This study showed the strong binding affinity of CNTs and fullerenes with all the tested targets presenting

it as a potential antiviral agent against SARS-CoV-2 and similar viruses (Skariyachan et al., 2021). Ag-TiO_2 single-atom nanozyme (SAN) has been studied as an antiviral agent in transgenic mice against SARS-CoV-2. The study revealed the adsorption capacity of Ag-TiO_2 SAN to the spike protein leading to phagocytosis of the virus-$AgTiO_2$ complex, thus blocking the replication of the virus (Wang et al., 2021b). A study presented nano-formulations against coronavirus in four types: the encapsulation of antiviral dyphylline in PEG-PLGA, graphene oxide sheets functionalized with AgNPs, Ag_2S nanoclusters, and Ag nanowires. All these types have shown an effective role against coronavirus by preventing its replication, blocking cellular endosomal acidification, and synthesis of viral RNA. Dyphylline is an ATPase blocker that showed replication inhibition of coronavirus in fcwf-4 cells in nano-formulation. For the treatment of COVID-19, the nanomaterials mimicking ACE2 receptors like AuNPs would be efficient as this receptor is responsible for the internalization and replication of SARS-CoV-2. The binding of AuNPs to the virus would potentially block its propagation in the cells (Alphandéry, 2020).

7 Conclusion and future implications of SARS-CoV-2 and the role of nanotechnology

7.1 Unsolved concerns of newly developing strains

Nanotechnology can be phenomenal in developing new strategies to tackle the emerging variants of SARS-CoV-2. The SARS-CoV-2 variants that have emerged because of the virus's S protein's ongoing changes have aggravated and prolonged the current pandemic, which the original SARS-CoV-2 first caused. Compared to earlier COVID-19 waves, the Omicron form has decreased hospitalization and mortality rates, despite its improved viral transmission and immune evasion (Bhattacharyya and Hanage, 2022). For this purpose, Johnson & Johnson, the two existing mRNA vaccine producers (Moderna and Pfizer-BioNTech), and the development of novel Omicron variant-specific vaccinations have all been disclosed (Thakur and Ratho, 2022). However, the effectiveness of these vaccinations has declined, considering the new SARS-CoV-2 variants. The Food and Drug Administration has consequently declared that these establish guidelines for expediting the assessment of updated vaccinations against specific variants. These variants can potentially be defeated by using innovative nanotechnology-based techniques that target the altered S protein of the virus. Due to their higher initial efficacy than traditional vaccinations, nanotechnology-based vaccines can still protect people from these variants. In addition, they can be quickly and easily updated to increase their effectiveness against the SARS-CoV-2 variants. This means that developing next-generation vaccines against SARS-CoV-2 variants will significantly aid nanotechnology-based solutions' extraordinarily high efficacy and rapid production cycle times. To create innovative nanotechnology-based therapies to battle SARS-CoV-2 variations, a range of nanostructures (for instance, DNA origami) that directly capture variants or accurately display neutralizing antibodies or ACE2 receptors on their surfaces might be modified. We anticipate that, despite the COVID-19 pandemic's continuous nature and the SARS-CoV-2 variants' quick worldwide spread, nanotechnology's innovations and advancements will enable various strategies to speed up the pandemic's termination.

7.2 Gaps that need to be filled

Some gaps need to be filled to make nanotechnology-based solutions to SARS-CoV-2 variants more effective. To this end, the pharmaceutical industry must work on research nano therapies to treat COVID-19, considering the potential presented by nanotechnology, nanomedicine, and biotechnology. From a pharmaceutical standpoint, developing stimuli-responsive, immune-supportive, and immunomodulating agents using nano-pharmacology principles, designing stimuli-responsive, immunomodulating, and immune-supportive antimicrobial/antiviral therapeutic agents with better efficacy and fewer side effects, optimizing dosages and delivery systems for carriers and targets and investigating biocompatible, bio-functionalized, nano drug loading systems are some of the undeveloped areas that need to be considered.

Similarly, there is an inherent risk associated with using nanostructures. The safety-to-risk ratio of nanostructures should be increased so that the present pandemic issue might be seen as an excellent chance to revolutionize nanomedicine. In-depth research, experience sharing, and information exchange across many businesses, regulatory bodies, and, ultimately, different nations are necessary for the ultimate result. Developing early-stage regulatory guidelines and conducting studies on opportunities and barriers to realizing nanotechnology-based solutions to SARS-CoV-2 variants is essential. This way, nanotechnology can help the world's medical community to fight current and subsequent pandemics effectively (Singh et al., 2021).

Finally, advanced machine learning and Artificial intelligence are other ways forward for proper and effective modeling and interpretation of cell-nanomaterial interactions, which are required to formulate quantitative nanostructure activity effectively. The effective use of nanomedicine in the pandemic

can be further complemented by other related technologies like robotics, telemedicine, and 3D printing (Miyazawa et al., 2021).

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Nano-antivirals: A comprehensive review

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Nanoparticles can be used as inhibitory agents against various microorganisms, including bacteria, algae, archaea, fungi, and a huge class of viruses. The mechanism of action includes inhibiting the function of the cell membrane/stopping the synthesis of the cell membrane, disturbing the transduction of energy, producing toxic reactive oxygen species (ROS), and inhibiting or reducing RNA and DNA production. Various nanomaterials, including different metallic, silicon, and carbon-based nanomaterials and nanoarchitectures, have been successfully used against different viruses. Recent research strongly agrees that these nanoarchitecture-based virucidal materials (nano-antivirals) have shown activity in the solid state. Therefore, they are very useful in the development of several products, such as fabric and high-touch surfaces. This review thoroughly and critically identifies recently developed nano-antivirals and their products, nano-antiviral deposition methods on various substrates, and possible mechanisms of action. By considering the commercial viability of nano-antivirals, recommendations are made to develop scalable and sustainable nano-antiviral products with contact-killing properties.

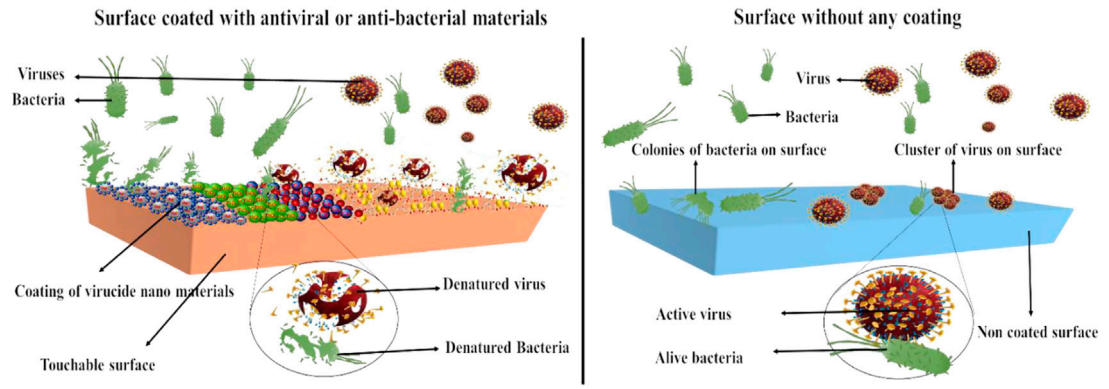
KEYWORDS

SARS-2, pandemic, contact-killing, virucidal solids, metallic nanoparticles, carbon based nanomaterials, silicon nanoparticles, self sanitizatio

1 Introduction

Viruses are the major causative agents for more than 60% of human infections worldwide. The most common viral diseases are produced by enteric and respiratory viruses. There are several ways of viral transmission from an animal or infected person to a new host. Recent research suggests that contaminated surfaces or fomites play a role in spreading viral diseases in humans. The dispersal of viruses due to contaminated surfaces depends on the potential of the virus to sustain its infectivity in the environment. However, infectivity can be reduced by any single factor or a combination of chemical, biological, and physical factors (Vasickova et al., 2010). Environmental disinfection has started to gain importance recently. Currently, it is included in several national and international infection control recommendations and policies.

Effective biocidal action for viruses, bacteria, and fungi is one of many criteria considered for any antimicrobial treatment. However, it is important to concentrate on



GRAPHICAL ABSTRACT

using less dangerous chemicals. There is urgent need to develop such materials with no or minimum effects on the skin, environmentally friendly, favorable for textile processing, durability, stability, sustain washing chemicals as well as withstand to hot pressing. Textiles are non-woven or woven products produced by synthetic or natural fibers. Textiles have a wide range of usage in different areas, such as clothing, the military, the food industry, home furnishings, sports equipment, the medical industry, building materials, and the automotive industry. Textile usage defines its properties, such as smoothness, waterproofness, breathability, degradability, elasticity thread count, temperature stability, and antibacterial and antiviral properties (Iyigundogdu et al., 2017). Due to their large surface area and ability to absorb and retain oxygen and water, textiles become a good host for the growth of many microorganisms. Furthermore, other factors facilitating the growth and multiplication of microorganisms are pH, nutrition, and temperature (Morais et al., 2016).

Because of the growing demand, there is good room for research on antiviral textile fabrics. Most of the antiviral fabrics reported in the literature have not been widely prepared at an industrial scale and have not been commercialized yet. Antiviral fabric textiles have various purposes and applications and are considered clean, safe, and hygienic fabrics. The health and public transport sector may be the main consumer market for antiviral textile fabrics. Antiviral fabrics could kill viruses on the surface, reducing the chances of biofilm formation (Von Borowski and Trentin, 2021). Thus, the chances of infection and reinfection are reduced. In general, antiviral fabric textile retains virucidal potential up to numerous wash cycles. Its reusable property can help minimize solid waste generation (Abou Elmaaty et al., 2022).

Viruses can be transferred in different ways, including 1) by other inanimate surface or living organisms, 2) from the accumulation of particulate aerosols, 3) by direct transfer from the contact of an infected person through secretion, and 4) by surface contact (Iyigundogdu et al., 2017). A thesis on norovirus detection and surface-to-surface transfer from the University of Helsinki concluded that HuNoV could be transmitted easily from human hands to eatable items and environmental surfaces. Therefore, to inactivate HuNoV, there is a need for proper hand hygiene and other effective measures, including UV, for virus management. The origin of viruses is normally very difficult to define due to sudden outbreaks, but it is necessary to take control measurements efficiently to minimize the deadly effect. Furthermore, none of the peripheral surfaces are completely “clean.” An organic substance layer is always present, and through contact with the animal or human skin, the surface will be contaminated with sebum components, microorganisms, and different molecules. A surface exposed to air, especially a horizontally exposed surface, will be covered with oily emulsions, powder particles, and aqueous aerosols. Examples of such surfaces are service counters in hospitals, railings of trollies, and patient beds. Furthermore, depending on its uses and surface structure, a structural-layered soil may contain different fungal spores, bacterial cells, spores, oils, and dirt. Evidence of the role of surfaces in the transmission of pathogenic microorganisms causing infections from healthcare is reported in detail in a consensus document by Morais et al. (2016). They have concluded that high-touch surfaces cause the spread or transmission of diseases, and disinfection should be considered a holistic approach.

At present, research on the persistence of veterinary and human coronavirus on inanimate surfaces and strategies for their inactivation with different biocidal agents for chemical disinfection is reviewed in health facilities. Reusable pathogen-contaminated medical devices can be a source of human infections. If these tools have to be reused, essential precautions must be taken before reuse for the next patient to prevent pathogen contamination. These measures are termed reprocessing in healthcare settings and include sterilizing, disinfecting, or cleaning medical devices (Patoo et al., 2022).

The scientific literature indicates that the contaminated surfaces and non-critical patient care processes play a significant role in the transmission of various health-related pathogens, including vancomycin-resistant *Enterococci*, methicillin-resistant *Staphylococcus aureus*, *Norovirus*, *Clostridium difficile*, and *Acinetobacter*. Therefore, the disinfection of the surfaces of medical devices and non-critical environmental surfaces is one of the strategies to prevent infection-causing pathogens and contamination (Jamunkar et al., 2022).

An analysis comprising twenty-two studies reveals that coronaviruses such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), or human coronavirus (HcoV) can persist up to 9 days on glass, plastics, or metals (Goharshadi et al., 2022). Moreover, 229E, a human coronavirus, will remain infectious in the human lungs' cell culture model for at least 5 days and persist on the range of some common non-biocidal surfaces, including polyvinyl chloride (PVC), PTFE, polytetrafluoroethylene (Teflon), glass, ceramic tiles, stainless steel, and silicon rubber (Góral and Góral-Kowalczyk, 2022). These contaminated surfaces can be effectively inactivated by surface disinfection procedures with 0.1% sodium hypochlorite, 62%–71% ethanol, or 0.5% hydrogen peroxide in 1 min or using 0.02% chlorhexidine digluconate or 0.05%–0.2% benzalkonium chloride, among others. In addition to manual cleaning with liquids, new technologies are emerging. Novel "non-touch" (self-cleaning) decontamination technologies, including aerosol and evaporating hydrogen peroxide emitting mobile devices, pulsed xenon UV light system, ultraviolet light (UV-C), and high-intensity narrow spectrum using 405 nm light. These "non-touch" automatic technologies can reduce bacterial contamination on different surfaces. The micro-condensation hydrogen peroxide system is related to minimizing colonization or reducing infection in multiple studies. However, there is limited evidence of infection reduction by a pulsed xenon system. A prospective randomized controlled trial has been recently completed, where continuous ultraviolet light (UV-C) is an efficient technology that can minimize healthcare-related infections and contaminations (Sondi and Salopek-Sondi, 2004). In addition, the concept of self-cleaning surfaces is developing, and new concepts are emerging in the literature. In hospital environments, high-contact self-sanitizing surfaces are recommended in place of aluminum or stainless steel. The use of copper instead of aluminum or stainless steel has shown clear advantages in the reduction of viral spread. Hydrophobic polymeric covalently applied to solid surfaces on metals, plastics, glass, polymers and fabrics, gauze, bandages, tissues, and other fibers are coated by brushing, spraying, or dipping to make surfaces virucidal and bactericidal are developed. Numerous material are available including hydrophobic, water-soluble polymers, charged and linear or branched polyethyleneimine. High molecular weight polymers are reported to be more virucidal. The dissolution and coating of these polymers by brush or spray is patented by Baram-Pinto et al. (2009). Nanomaterials are emerging antivirals (Patoo et al., 2022). A review of the antiviral activity of silver nanoparticles was reported by Jamunkar et al. (2022), and the role of nanotechnology as a whole is reviewed by Goharshadi et al. (2022).

Viral transmission has many modes, and many studies (even pre-SARS-CoV-2-pandemic) support the role of surfaces in cross-contamination and the spread of viruses. The field of antiviral surface coatings is evolving rapidly, witnessing a large increase in 2021 (Góral and Góral-Kowalczyk, 2022). By considering the emergence of pandemics and the role of virucidal materials, this review summarizes reports on new groups of viral compounds, nanoparticles, compositions, or surface coatings that can be effectively transformed into self-cleaning products. Furthermore, inconsistencies in the analysis of the viral activity of solid surfaces have recently been reported, so a brief overview of the literature that has arisen in the analysis of viral activities is also provided.

2 Nano-antiviral mechanism of action

Nanoparticles show excellent antiviral activity toward many strains of viruses because of their unique properties. Silver nanoparticles are widely studied because of their incredible activities. The antioxidants, anti-inflammatory, antiangiogenic, anticancer, antimicrobial, antifungal, and antiviral activity of silver nanoparticles has been widely studied (Sondi and Salopek-Sondi, 2004; Kalishwaralal et al., 2009; Al-Shmgani et al., 2017; Deyá and Bellotti, 2017; Singh et al., 2018; Yuan et al., 2018; Nagarajan et al., 2019). The mechanism of action of metal ions or metal nanoparticles can be the attachment or connection of nanoparticles to the surface of the virus, making the viral cell unable to be attached to the host cell; the production of highly reactive oxygen species that could attach to the spike or membrane of virus and inhibit the function of nucleic acid and protein; the enhancement of the immune system of the host by stimulating nucleus; and the destruction of the host cell and the inhibition of virus spread (Figure 1).

Silver nanoparticles are highly effective against viruses including influenza, HIV-1, monkeypox, herpes hepatitis B, respiratory syncytial, simplex, and Tacaribe viruses (Sun et al., 2005; Rogers et al., 2008; Baram-Pinto et al., 2009; Lara et al., 2010a; Papp et al., 2010; Speshock et al., 2010; Lara et al., 2011). In the case of HIV-1, silver nanoparticles get attached to the sulfur of gp120 (a spike protein present on the viral membrane), thereby blocking virus attachment to the host cell membrane (Lara et al., 2010a). The viral entry for SARS-CoV-2 into the host cell depends on proteases that divide angiotensin-converting enzyme 2 (ACE-2), a protease and receptor spike proteins, which are receptors of SARS-CoV-2. The life cycle of SARS-CoV-2 begins when spike protein binds to ACE-2. The ACE-2 has a very important lysine 31 residue recognized as critical in

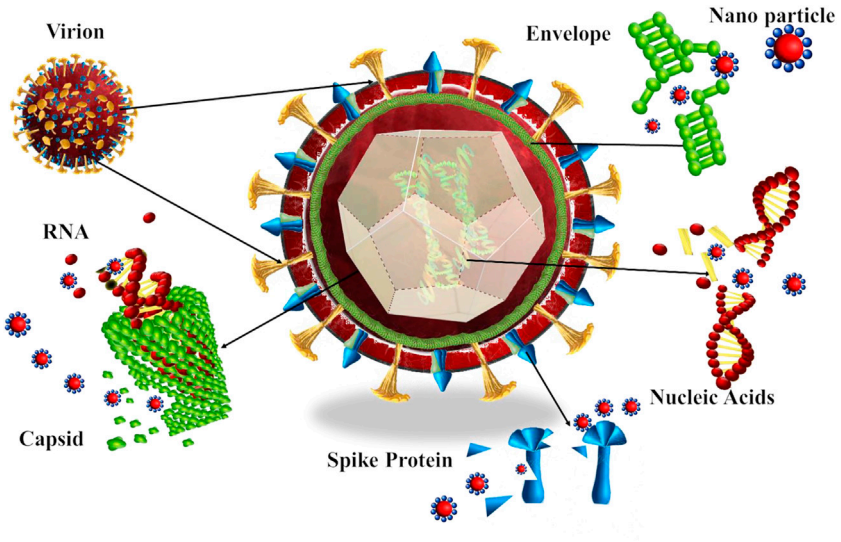


FIGURE 1
Antiviral mechanism of typical metal nanoparticles. Viruses generally infect the host cell through four mechanisms: 1. attachment to the host cell, 2. penetration, 3. viral replication, and 4. budding. The metallic nanoparticle inactivates viruses by attachment to the virus, inhibiting the attachment virus to the host cell, damaging the peptidoglycan bonds, increase levels of H2O2, O2- or OH-, which are reactive oxygen species (ROS), binding to the spike of cell in order to stop attachment, destruction of cell structure, functional and structural proteins, destruction of nucleic acid and promote host immune system to inhibit the spreading and budding of the virus.

binding with 394 glutamine residues present in SARS-CoV-2 (Bertram et al., 2011; Glowacka et al., 2011; Shereen et al., 2020). Therefore, blocking the 394-glutamine residue in spike protein by silver nanoparticles may play a role in inhibiting the viral entry into the host.

Another report suggested that silver nanoparticles act as an entry inhibitor or virucide toward HIV at the initial stage of the viral cycle. A specific interaction between silver nanoparticles and HIV is formed by binding with gp120. Silver nanoparticles, particularly in the case of HIV, block the entry of the virus by gp120-CD_4 interaction through binding with the protein structure of the viral membrane. The interaction of silver nanoparticles with gp120-CD_4 is generally electrostatic (Lara et al., 2010a). Additionally, silver nanoparticles may interact with disulfide bonds present in the carboxyl of the HIV-gp120 glycoprotein. As a result, silver ion reduces the disulfide bonds and denatured proteins of the virus. Additionally, silver ions can form a complex with oxygen, sulfur, and nitrogen (electron donor groups), which are generally present as phosphate or thiols on the nucleic acid and amino acid of the virus. These particles can also directly bind to DNA or RNA and reduce the transcription rate (Lekutis et al., 1992; McDonnell, 2007; Varni et al., 2007; Lara et al., 2010a). Another suggested route in the literature for the deactivation of the virus using nanoparticles is the size of nanoparticles, which plays an important role in the antiviral activity. The interaction of silver nanoparticles with glycoprotein present in the spike of virion of HSV-2 shows that the center-to-center space of the spike is about 9–13 nm, and the height of spikes is about 10–25 nm. Hence, small-sized nanoparticles of silver (i.e., 13 nm) have a greater binding affinity compared to the size of 33–46 nm. The shape of nanoparticles is reported to be an important factor in viral inhibition. For instance, cubic, cylindrical, or spherical shapes have less cellular attachment in the case of HeLa cells compared to the rod shape nanoparticles (Orlowski et al., 2014; Banerjee et al., 2016; Alavi et al., 2022).

Besides silver, zinc nanoparticles are also reported as antivirals. Zinc is part of various biological activities, including the expression and activity of different transcription factors and cellular enzymes. Zinc also shows great antiviral potential through various mechanisms. Zinc is a cofactor of different viral proteins and helps in misfolding viral polyproteins by facilitating the proteolytic process through misfolding, which changes the structure of the virus (Te Velthuis et al., 2010). Zinc may inhibit the Semliki Forest virus by inhibiting membrane attachment. Zinc prevents virus entry into the cell by binding with the histidine protein of the Semliki Forest virus at glycoprotein E_1 (Kümel et al., 1990; Te Velthuis et al., 2010; Liu and Kielian, 2012; Ishida, 2019; Rani et al., 2021). Another study reported that zinc inactivates the varicella-zoster virus (Shishkov et al., 1996). In many viruses, including HIV, rhinovirus, vaccinia virus HSV, and SARS-CoV, zinc helps kill the virus by stopping its entrance, RNA-dependent RNA polymerase, or polyprotein processes (Te Velthuis et al., 2010). Zinc also plays a role in inhibiting the replication of alphavirus and flavivirus and enhances oxidative stress (Rani et al., 2021).

Copper acts differently on different types of viruses. For example, in the case of the coccolith virus, copper disturbs the lytic cycle by increasing the generation of reactive oxygen species (Haldar et al., 2007). Copper cross-links and binds with the strands of DNA and destroys the viral genome. The contact killing time for copper is suggested as 7–8 logs/h, which means that the virus gets destroyed exponentially 1×10^7 times in an hour (log 3/h reduction is considered 100% as ISO 18184 classification). When copper is contacted with the virus's surface, copper intervenes in the virus as the nucleic acid of the virus degrades. Copper inactivates the virus by changing its viral genomes through damaging RNA and causing structural changes, which include rupturing of the envelope and surface spike dispersal. Van Doremalen et al., 2020 compared the stability of SARS-CoV-1 with SARS-CoV-2. Results showed that SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard, and a viable virus was detected up to 72 h after application to these surfaces. On copper, no viable SARS-CoV-2 was measured after 4 h and no viable SARS-CoV-1 was measured after 8 h (Van Doremalen et al., 2020). In general, copper nanoparticles create oxidative stress, which can disassemble bacterial or viral membranes. Thus, it can interfere with their viral activity. Furthermore, copper ions facilitate the generation of compounds (e.g., OH radicals and redox actives) that are toxic to microbes. On another side, copper nanoparticles may change the homeostasis of the cell through metal ions. If a cell is exposed to a concentration higher than normal, the system of the cell ultimately collapses (Ermini and Voliani, 2021). However, this mechanism may or may not be applicable for viruses as these infectious agents live in a dynamic equilibrium (homeostasis) with their hosts in which both immune and non-immune pathways contribute to viral homeostasis. However, the disruption of these pathways can have dramatic consequences on pathogenesis (Von Borowski and Trentin, 2021).

Few studies have been conducted on the antiviral activity of quantum dots. Moreover, a few showed that quantum dots exhibited good antiviral activity. Some studies showed that modified quantum dots could inhibit virus entry into most cells by modifying the structure of proteins. The attachment of quantum dots to the membrane reduces the virus quantity and effectively inhibits the multiplication of viral RNA (Du et al., 2016; Du et al., 2018; Gurunathan et al., 2020). In the case of graphene oxide or its derivatives or graphene-based nanoparticles, lateral size plays an important role in the antiviral activity. Sharp edges, adsorption, and desorption are largely affected by the particle size of the material. Studies have revealed that higher lateral size and greater absorption ability attributed to greater surface energies and antimicrobial properties were greater with large-size graphene oxide sheets compared to smaller ones (Cai et al., 2011; Jabbar et al., 2020). Antiviral activities of graphene oxide (large sheets) and reduced graphene oxide (relatively smaller sheets) are significantly different, probably owing to size variations. Other factors include the number of layers present in graphene, which increases its thickness and weakens the nano-knife effect, increases aggregation, decreases dispersion that results in less contact between microbes and materials, and alters the antimicrobial effects (Wang et al., 2013; Zou et al., 2016; áde Leon et al., 2015). Graphene oxide and reduced graphene oxide carry a negative charge, helping them attract viruses with a positive charge, henceforth higher charge attributes more attraction as the graphene oxide has a higher charge compared to the reduced graphene oxide, so it has more affinity to attract virus (Mohammed et al., 2020; Nasrollahzadeh et al., 2020). Fabrication of functional groups, including carboxyl hydroxyl and carbonyl, can amplify the redox reaction between graphene oxide and viruses. The graphene and reduced graphene oxide have an intrinsic capacity to absorb lipids due to their charge, which promotes the destruction of the viral membrane. It has been observed that the feline coronavirus's lipids bilayer was absorbed on the surface of graphene and the reduced graphene oxide owing to the electrostatic interaction between the negatively charged group in the graphene oxide and the positively charged viral particles, which made it difficult for the virus to bind (Chen et al., 2016). Another important factor that plays in viral inactivation is particle shape. It has been observed that sharp edges of graphene oxide nano-walls and reduced graphene oxide nano-walls inactivate the attachment and inhibit the entrance of viruses before they get into the cell, which is done by destroying the outermost layer. Thus, the morphology of the virus will be changed, and their functions got disturbed (Mohammed et al., 2020). Functionalities of graphene oxide or reduced graphene oxide play a significant role in preventing agglomeration and, hence, their antiviral properties. Antifouling property enhancement can improve the function of antiviral coatings and surfaces (Ayub et al., 2021).

3 Virucidal compositions for solid surfaces

The most common surface coatings for virucidal activity, as reported in the literature, are 1) based on polyethylene-imine or polyethylene-amine type compounds, which are polyelectrolytes, and 2) metal salts and oxides. The supporting materials (substrates) are mostly metallic surfaces, fabric, and glass. Coatings can be accomplished by selecting polyelectrolytes and controlled conditions for the deposition. Multilayers can be deposited on the surface for coating with carefully controlled adhesive properties according to the targeted viruses (Hsu and Wu, 2019). The results obtained from MS2-type bacteriophage (MS2) adhesion using quartz crystal microbalance with the degeneracy monitoring were compared with the predictions by the (XDLVO) Derjaguin–Landau–Verwey–Overbeek

theory. Different surfaces show different kinetics and variable capacities for the deposition of MS2, indicating the potential of polyelectrolyte for multilayer deposition, a promising and easy method to apply coating on the surface for adsorption of viruses on the surface, inactivation of the virus by the viricidal properties of cationic polyelectrolytes, and minimization of the exposure of viruses to humans (Dang and Tarabara, 2019). Another ionically crossed-linked polymeric film was introduced using the layer-by-layer (LbL) technology, and silicon was used as a substrate in order to increase contact killing property of surfaces. N,N-dodecyl,methyl-polyethylenimine (polycation) with antimicrobial activity was layered along with poly acrylic acid (polyanion) using the LbL technology to create a film. This surface is highly effective against airborne and waterborne *Staphylococcus aureus* and *Escherichia coli* (Gram-positive and Gram-negative bacteria, respectively). It shows great results against A/WSN (H1N1) influenza viruses (Wong et al., 2010).

Another antiviral material was developed using cationic polyallylamine (PA) polymer bonded covalently with glass. This PA film showed excellent antimicrobial activities against Gram-negative and Gram-positive bacteria, including *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Iarikov et al., 2014). Moreover, polycation N, N-dodecyl,methyl-polyethylenimine is coated on glass slides along with the solution of polycations butanol. The results showed a 100% reduction in *Escherichia coli* and *Staphylococcus aureus* and strains of the influenza virus (Deyá and Bellotti, 2017). Another multistep synthesis of the antimicrobial surface is reported by replacing hydrophobic polycations with an aerosol-assisted plasma deposition procedure, in which HMPEI (N,N-hexyl,methyl-PEI) is coated (plasma-coated) directly on glass surfaces. This coated surface has shown robust results against *Escherichia coli* and the human influenza virus (Liu et al., 2014). A paint-based application of polycation antibacterial surface has been investigated in detail for its practical applications. The authors claim stability of the coating process over the impregnation process (Mukherjee et al., 2008). N,N-Dodecyl,methyl-polyethylenimines (PEIs) were coated on glass, and their efficacy was tested for H1N1 and H3N2, found as 100% within 30 min and 2 h, receptively (Haldar et al., 2006).

Metallic nanoparticles are deposited directly by adsorption, spray, or dip-coating or using linkers and by sonochemical or magnetron sputtering methods (Góral and Góral-Kowalczyk, 2022; Meister et al., 2022). Zinc citrate was coated on muslin cloth, which showed 99.7% virucidal activity for H3N2 (Nonomura, 2007). The antiviral activity of four compounds, namely, $CuCl_2$ (copper ionic compound), Cu_2O and CuO (solid-state copper compounds), $AgNO_3$ (silver ionic compounds), and Ag_2S, and Ag_2O (a solid-state silver compound) have been investigated against the bacteriophage Qβ and surface protein of influenza A viruses, neuraminidase (NA), and hemagglutinin (HA). Cu_2O (solid-state copper) has shown itself to be a promising candidate against non-enveloped and enveloped viruses compared to silver compounds because it has a unique mechanism of inactivation supported by direct contact (Minoshima et al., 2016; Sunada et al., 2012). New and durable platform technologies have been invented to introduce copper into latex, polymeric materials, and cotton fibers. This technology helps produce antiviral filters and gloves that can deactivate viruses, including HIV-1; self-sterilizing antibacterial fabrics that can kill antibiotic-resistant bacteria, including vancomycin-resistant *Enterococci*, and methicillin-resistant *Staphylococcus aureus*, anti-dust mite mattress covers to reduce material allergies and antifungal socks, which can help reduce the symptoms of Athlete's foot (Borkow and Gabbay, 2004). The same researchers have developed copper oxide-impregnated fabric for various uses (Borkow and Gabbay, 2004). Recently, a copper sulfate hybrid with alginate using ceramic as a substrate has been reported for the inactivation of CoV-2 (Bataglioli et al., 2022). Furthermore, the copper oxide-impregnated face mask was approved by Occupational Safety and Health Administration (OSHA) (United States) and is now used in general practice for safety (Borkow et al., 2010).

A new mode of incorporation of virucidal material was reported where copper glass (copper oxide incorporated aluminosilicate) powder was dispersed in latex paint and tested as a durable virucidal surface coating (Gross et al., 2019; Hodek et al., 2016). The coatings were tested using EPA recommended procedure for solid surfaces and found to be comparable with metallic copper.

3.1 Metals as virucides

Metallic nanoparticles have gained the interest of researchers due to their unique properties, including small size and high specific surface areas that can help interact with microorganisms, such as bacteria and viruses (Birkett et al., 2022). Otherwise, nanoparticles can enhance cytokine production and induce a hormonal immune response and have the capacity for cell recruitment, biocompatibility, low toxicity, good biodistribution properties, and chemical inertness (Dykman, 2020; Behzadi et al., 2021; Abate et al., 2022). Several metals and metal oxide nanoparticles, including copper, silver, gold, zinc, and titanium, have been studied as antiviral agents.

3.1.1 Copper and its nanoparticles

Copper is long known for its antimicrobial applications (Haldar et al., 2006; Grass et al., 2011; Steinhauer et al., 2018), whereas some studies have shown equal potential for its alloys. Copper alloys are more active antivirals compared to pure copper. Compared to other metals, such as platinum, gold, silver, and palladium, copper is the cheapest and most easily available (Grass et al., 2011). Another advantage of copper is that it gets oxidized to form copper oxide nanoparticles that can be blended with different

macromolecules or polymers and give a stable product in terms of physical and chemical properties (Cioffi et al., 2005). Copper alloys are effective in inactivating murine norovirus by destroying the RNA genome when it is exposed to copper surfaces. A study suggested that copper ions were still indirectly or directly responsible for inactivation, but it has not created reactive oxygen species (ROS) as a toxicity mechanism. Although their mechanism of destruction can vary and can be suggested as multimodal, it is noteworthy that copper alloys are very promising candidates for the destruction of various disease-causing microorganisms (Haldar et al., 2007; Ikner et al., 2020). Besides copper and its alloys, efforts are made to develop coatings for inanimate metallic and non-metallic surfaces to cover the high-touch surfaces, which are prone to soiling (accumulation of dirt, microbes, etc.). Alloys of copper, nickel, and zinc are tested against viruses to replace steel as high-touch surfaces (Bregnocchi et al., 2022). The copper alloy was shown to have a strong virucidal activity under clean and moderate soiling conditions (>four-log reduction) for virus droplets or dried virus onto the surface. Multiple exposures of the surface to viruses indicate that the surface could not inactivate virus droplets (three-log), regardless of no or moderate soiling. Heavy soiling reduced inactivation below an acceptable efficacy threshold. Virucidal tests of copper, nickel, and zinc ions indicate that copper and nickel were significantly virucidal (Konieczny and Rdzawski, 2012). Copper has been known as a biocide since 200 BC and has been extensively used in ancient times (Grass et al., 2011; Konieczny and Rdzawski, 2012). Recently, various compositions of copper have been used for the treatment of anemia, facial neuralgia, chorea, tubercular infections, adenitis, impetigo, scrofulosis, eczema, syphilis, and lupus (Grass et al., 2011). Copper is effective against various types of viruses, including poliovirus, HIV-1, hepatitis C, monkeypox, murine norovirus, herpes simplex, bronchitis, and COVID-19 (Cortes and Zuñiga, 2020; Gauri et al., 2020). The antiviral activity of copper (II) chloride dihydrate solution against the dengue virus type-2 *in vivo* cell was also reported (Sucipto et al., 2017). Compared to silver, Cu_2O has shown a good result against unenveloped and enveloped viruses (Jung et al., 2021). At 2.5–250 μM concentrations over time, Cu^{2+} can inhibit the H9N2 virus, which infects the MDCK cells. In the 25 μM Cu^{2+} solution, the virus titer decreased by about three and four logs during 3–6 h. Compared to Cu^{2+}, Zn^{2+} was much less effective against virus inactivation. At 2.5–250 μM, Cu^{2+} did not affect the activity of the H9N2 virus hemagglutinin nor the neuraminidase (NA). This shows that copper ions suppress the infectivity of the influenza virus at lower concentrations at which neither NA nor hemagglutination inhibition occurs. Therefore, the mechanism of action requires further studies (Horie et al., 2008; Jana et al., 2021). Copper creates toxicity to microorganisms by various parallel mechanisms that can cause cell death even immediately after minutes of exposure to copper. The first area damaged by copper can be the envelope of microorganisms. Copper-containing steel was found to adhere to the bacterial plasma membrane by exerting the electrostatic forces by Cu^{2+}. Copper may also cause structural changes in protein structure. For example, the oxidation of cysteine in the active region of the protein tyrosine phosphatase associated with the Cu^{2+} active vaccine H1 resulted in complete inactivation of the protein activity. Copper ions can also damage nucleic acids by cross-linking between and within DNA chains. The mechanism can be considered oxidative damage or like Fenton's reaction. In general, the redox cycle between Cu^{2+} and Cu^{+} may be associated, which can catalyze the production of highly active hydroxyl radicals and then damage proteins, DNA, lipids, and other biomolecules (Papp et al., 2010). Shionoiri et al. (2012) used copper iodide nanoparticles (CuI)NP against feline calicivirus, a non-enveloped virus used as a human norovirus proxy. They found that virus infectivity reduced up to seven times at the dosage of 1,000 μg/ml CuI nanoparticles (Shionoiri et al., 2012). Purniawan et al. (2022) synthesized copper nanoparticles from copper sulfate with a diameter of 254 nm, mixed them with resin-based paint, and used them as a spray for surface coating agents against SARS-CoV-2. The result shows that within 10 min, the antiviral activity of the spray reaches 90%, 97.8%, and 99.99% after 10, 30, and 60 min, respectively. However, paint alone reached 97.8%, after 18 h of exposure time for inactivation of virus. Hutasoit et al. (2020) reported the coating of copper using the cold spray technique to fabricate copper on steel parts against SARS-CoV-2. The results showed that about 96% of SARS-CoV-2 get inactivated within 2 h. In another study, Behzadinasab et al. (2020) fabricated a coating using Cu_2O bound with polyurethane and applied it to steel or glass. The virucidal activity was 99.9% compared to the uncoated glass or steel. The coating material remained active for 13 days after various washing cycles with a mixture of 70% ethanol–water mixture. The coating is so strong that it cannot be removed with a razor blade. Bello-Lopez et al. (2021) reported a biocide effect against SARS-CoV-2 and ESKAPE pathogens of a non-cytotoxic silver–copper nanofilm. A nanometric layer of bimetallic AgCu was effectively deposited on polypropylene (PP) fibers. The virucidal results show more than a 95% reduction in viral load after 2 h of contact time (Bello-Lopez et al., 2021). El-Nahhal et al. (2012) reported CuO and CuS nanoparticles coated onto cotton fabric. Accordingly, CuO nanoparticles showed excellent antibacterial activity. However, CuS nanoparticles did not show any activity. Au/CuS based nanoparticles are reported as virucides are reported and recommended generally that inactivation by nanoparticles (NPs) is a universal alternative to other chemical and physical strategies that exhibit variable efficacy (Gurunathan et al., 2020). All the above discussion shows that copper is the antidote for viruses as it has the potency to deactivate viruses *in vivo* and *in vitro*. Based on scientific data, a patent entitled "copper-containing dry disinfection apparatus" was registered (Rogers et al., 2008). In addition, copper oxide nanoparticles have been reported as an antibacterial that can be extended to solid surfaces in suspension (Lara et al., 2011).

3.1.2 Silver-based nanoparticles

Silver nanoparticles have been successfully used against different types of respiratory viruses, including coronaviruses, rhinovirus, influenza virus, adenoviruses, hepatitis B virus, human immunodeficiency virus, and herpes simplex virus (Lara et al., 2010a; Galdiero et al., 2011; Demchenko et al., 2022; Parvez et al., 2022; Patoo et al., 2022; Zhang et al., 2022). Before the discovery of penicillin in the 1930s, silver was known as an antimicrobial for centuries. Due to their impressive antimicrobial activity, silver and silver-based nanoparticles still have very high demand. In addition, silver has been used to prevent infection and food spoilage (Hoyme, 1993). Besides the antibacterial and antiviral activity, silver nanoparticles are being extensively studied due to their stability, non-toxic nature, disinfectant capacity, water purification properties, high quantum efficiency, increased conductivity, anticancer biochemical capacity, and easy synthesis process (Jiang et al., 2005; Basheer et al., 2013; Cornelis et al., 2013; Bhosale and Bhanage, 2015; Rasool and Lee, 2016; Pandiarajan and Krishnan, 2017; Loo et al., 2018; Mahdi et al., 2018; Deshmukh et al., 2019; Shrivas et al., 2019; Das et al., 2020a; Ahn and Park, 2020; Das et al., 2020b; Gomathi et al., 2020). In the case of bacteria, silver ions inhibit the growth of bacteria by suppressing electron transport components and respiratory enzymes and interfering with the function of DNA. In addition to the antimicrobial and antibacterial activity, silver nanoparticles have been widely used in coating different medical devices, wound dressing, and textiles to prevent microbial growth and infection. One of the main advantages of using silver nanoparticles is their ability to continuously release silver ions, which increases their antimicrobial ability. The silver nanoparticles have been efficient against more than 650 types of microorganisms, including a wide range of bacteria, fungi, and viruses (Dakal et al., 2016). Generally, silver nanoparticles prevent virus attachment onto the host cell by interacting with the outermost layer of the bacteria. The physiochemical properties, including particle size, surface area, and the shape of nanoparticles, play an important role in antiviral ability. The antiviral activity of silver nanoparticles has been studied against a wide range of viruses including hepatitis B and C virus, influenza virus, poliovirus, dengue virus, herpes simplex virus, chikungunya virus, human immunodeficiency virus, Rift Valley fever virus, respiratory syncytial virus, vaccinia virus, white spot syndrome Virus, African swine fever virus, enterovirus, murine norovirus, porcine reproductive viruses, feline calicivirus, monkeypox virus, respiratory syndrome virus, Tacaribe virus, porcine epidemic diarrhea virus, tobacco mosaic virus, bacteriophage MS2, UZ1, φX17433, bean yellow mosaic, and coronavirus (Elechiguerra et al., 2005; Lu et al., 2008; Rogers et al., 2008; Sun et al., 2008; Speshock et al., 2010; De Gusseme et al., 2011; Gaikwad et al., 2013; Trefry and Wooley, 2013; Xiang et al., 2013; Elbeshehy et al., 2015; Sujitha et al., 2015; Borrego et al., 2016; Li et al., 2017a; Castro-Mayorga et al., 2017; Huy et al., 2017; Park et al., 2018; Ochoa-Meza et al., 2019; Sharma et al., 2019; Ahsan, 2020; Du et al., 2020; Dung et al., 2020; Jeremiah et al., 2020; Shady et al., 2020; Ghosh et al., 2022). Bekele et al. (2016) investigated the antiviral activity of silver nanoparticles with various dosages and sizes to evaluate the antiviral activity of feline calicivirus. The study reveals that the silver nanoparticles with diameters of 10 nm can successfully decrease the virus load because the virus size ranges from 27 to 40 nm, so the smaller size of nanoparticles can easily interact with the virus. Sundararaj Stanleyraj et al. (2021) evaluated the antiviral activity of silver nanoparticles on SARS-CoV-2. The results revealed that silver nanoparticles with a size of 10 nm could successfully inhibit the extracellular SARS-CoV-2. Jeremiah et al. (2020) reported the antiviral activity of silver against SARS-CoV-2. The size of the synthesized nanoparticles is 10 nm, and a dosage of 1–10 ppm prevents viral infiltration (Rahman et al., 2021). In another study, silver nanoparticle-fabricated textiles were tested against the influenza A virus and calicivirus and showed a promising result (Seino et al., 2016). Demchenko et al. (2022) studied the antiviral activity of silver coating polylactic acid chitosan polymer film against adenovirus serotype 2 and herpes virus type 1. The results revealed that 4% of synthesized nanocomposites have high antiviral activity against herpes virus type 1. The inhibition of the cytopathic effect against the virus reached 5.12 $\log_{10}TCID_{50}$/ml, whereas that against adenovirus and influenza virus reached 1.07 and 0.60 $\log_{10}TCID_{50}$/ml. The nanocomposite is ineffective against Hep-2, BHK-21, MDCK, and cell cultures. If the concentration of silver decreases by less than 4%, the antiviral activity also decreases. Nanocomposite contains 1% silver and does not show cytopathic action against adenovirus. In contrast, as the concentration increases to 2%, inhibition starts at 0.18 $\log_{10}TCID_{50}$/ml, whereas the maximum inhibition is achieved at 4% of the silver nanocomposite. Fetouh et al. synthesized silver nanoparticles with activated carbon by photodeposited silver nanoparticles into activated carbon. The composite showed good *in vitro* antiviral activity against the hepatitis A virus. In another experiment, silver nanoparticles were used to determine the *in vitro* antiviral effect on adenovirus type 3 (Ad3). The synthesized nanoparticles were fabricated through the redox method using 1% tannic acid in the silver nitrate solution. The results show a remarkable inhibitory effect against adenovirus type 3 (Ad3) due to possible DNA damage and the destruction of the structure of adenovirus type 3 (Chen et al., 2013). AgNPs have been used for the *in vitro* and *in vivo* inhibition of fungi, bacteria, and viruses. Particularly, in some reports, researchers used this nanoscale material to inhibit the coronavirus family, such as H1V1 and H3N2 influenzas. However, some reports debated the clinical trial or the clinical use of AgNPs for various treatments. Moreover, some reviews mentioned that the silver element was applied as an effective agent for various treatments in ancient medicine. It is suggested that at least the potential of silver nanoparticles and many non-

TABLE 1 Applications of various metallic nanoparticles in the deactivation of viruses.

Synthetic method	Particles size	Target specie	Size/ diameter of target specie	Time	Dosage	Reduction	Ref.
Copper nanoparticle-based polymeric spray	25 nm	SARS-CoV-2	≈0.1 μm	30 min	1% of CuNP	99% reduction	Foffa et al. (2022)
copper–graphene (Cu–Gr) nanocomposite	–	Influenza A virus, strain A/H1N1/WSN/ 1933	80–120 nm	2 h	5 μM	64%	Das Jana et al. (2020)
Copper oxide nanoparticles (radiolytic method)	100 nm	H1N1 virus	80–120 nm	30 min	f 0.5% w/v	Reduce viral load	Ha et al. (2022)
AgNPs capped with citrate, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) mercaptoacetic acid (MAA), and branched polyethyleneimine (BPEI)	10 nm	MS2 bacteriophages	23–28 nm	30 min– 24 h	0.01 mg ml^{-1}	Ag/BPEI reduction in ≥ 6 log_{10}, 4–5 log_{10} unit reduction with PVP and PEG and 3–4 log_{10} MAA and citrate capping	Sinclair et al. (2021)
Iron oxide nanoparticles	10–15 nm	H1N1 influenza virus strain (PR8-H1N1)	80–120 nm	72 h	1.1 pg	50% cell viability (TD50) was observed at 4.25 ± 0.2 pg	Kumar et al. (2019)
Silver nanoparticles using *Cyperus rotundus* L. extract	11–19 nm	laryngotracheitis virus (ILTV)	80–100 nm	–	400 μg ml^{-1}	IC50 310 μg ml^{-1} infection percentage 41.07% ± 0.9%	Abo-El-Yazid et al. (2022)
Silver nanoparticles using *Cyperus rotundus* L. extract	11–19 nm	Infectious bronchitis virus (IBV)	≈80 nm		400 μg ml^{-1}	95.20% ± 0.20%	Abo-El-Yazid et al. (2022)
Silver nanoparticles	50 nm	SARS-CoV-2	0.1 μm	24–48	10 μg/ml		He et al. (2022)
Silver nanoparticles coated with PVP	30–50 nm	HIV-1	≈100 nm	20 min	0.44–0.91 mg/ml	98% inhibition	Lara et al. (2010b)
Silver nanoparticles capped with mercaptoethane sulfonate	4 ± 1 nm	HSV-1	155–240 nm	24–72 h	400 μg/ml	97% decrease	Baram-Pinto et al. (2009)
ZnO nanoparticles supplemented with geraniol and carvacrol	–	6 bacteriophages	≈75 nm	16 h	Coating containing 0.041 g ZnO nanoparticles and 0.0125 g carvacrol in 100 ml	Moderate activity against 6 phage	Mizielińska et al. (2021)
Zinc oxide nanoparticles	5–500 nm	Coronavirus	≈0.1 μm	10 min	–	Reduction 99.9%	Gonzalez et al. (2021)
ZnO nanostructures		SARS-CoV-2	≈0.1 μm	–	1 g/L	Reduction up to 70% and 90%	Sportelli et al. (2022)
Tannic acid-modified silver nanoparticles	13–46 nm	HSV-2 strain 333	≈160 nm	–	0.5 and 1 μg/ml	95% ± 1.38% and 95.4% ± 0.42%, respectively	Orlowski et al. (2014)
Silver nanoparticles	7.1 nm	Poliovirus	25–30 nm	60 min	3 μg/ml and	98%	Huy et al. (2017)
Porous gold nanoparticles	140.23 ± 25.10 nm–154.24 ± 37.05 nm	Influenza A virus (H1N1, H3N2, and H9N2)	80–120 nm	60 min	0.2 mg/ml	Antiviral activity up to 74%, 76%, and 56%, respectively	Kim et al. (2020)
Gold nanoparticles (GAuNPs)	7 nm	Herpes simplex virus (HSV)	155–240 nm	–	32.3 μM	CC_{50} 972.4 μM	Halder et al. (2018)
Silver nanorods	–	HIV and HSV viruses	≈100 nm and ≈160 nm	–	10 μmol/ml	90% of HSV virions failed to replicate	Etemadzade et al. (2016)
Copper iodide nanoparticles	–	Virus of swine origin (H1N1)	80 –120 nm	60 min	17 μg/ml	50% reduction	Fujimori et al. (2012)

hazard nano-metals and nano-metal oxides (by special dosages) could be considered (by related scientists) as candidates for the inhibition of 2019-nCoV (Du et al., 2018). Implantation of metal-containing nanoparticles on the surface of the metal oxide cover layer as germicidal has been reported. The plasma method was utilized according to the invention to produce the nanoparticles, which permits the extensive immobilization of the nanoparticles and, therewith, the control of the dosing of the metal ion release and the minimization of the risk of mobility of nanoparticles. The germicidal action of the metal ions without light exposure is of great significance in medicine (Du et al., 2016).

3.1.3 Zinc-based nanoparticles

Zinc is naturally present in a variety of dietary items. Zinc is also known for its antiviral activities against different viruses, including severe acute respiratory syndrome coronavirus (SARS CoV), human immunodeficiency virus (HIV), human papillomavirus (HPV), hepatitis C virus (HCV), hepatitis E virus (HEV) rhinovirus, respiratory syncytial virus (RSV), herpes simplex virus (HSV), and equine arteritis virus (EAV) (Korant et al., 1974; Haraguchi et al., 1999; Suara and Crowe, 2004; Te Velthuis et al., 2010; Kaushik et al., 2017). Gupta et al. (2022) studied the antiviral activity of zinc oxide nanoparticles and tetrapod-shaped zinc oxide nanoparticles against hepatitis C and hepatitis E viruses. The synthesized zinc oxide nanoparticles successfully inhibit viral replication. The study revealed that, at a considerable dosage of zinc oxide, nanoparticles are non-cytotoxic to cells. Ghaffari et al. (2019) used zinc oxide nanoparticles modified with polyethylene glycol and tested them against the H1N1 influenza virus. The size of synthesized nanoparticles was estimated between 16 and 20 nm. The results show viral inhibition up to 94.6%. Melk et al., 2021 synthesized zinc oxide nanoparticles mediated by the extract of *Plumbago indica* L. The synthesized particle size was 2.56–8.83 nm, applied to an antiviral test against herpes simplex virus type 1(HSV-1). The results show that IC_{50} and CC_{50} are equal to 23.17 ± 2.2 μg/ml and 43.96 ± 1.39 μg/ml, respectively (Melk et al., 2021). Zinc basically module the immune response to stop virus replication. It is a mediator of lipopolysaccharides in bacteria (LPS) and toll-like receptor 4-(TLR_4-) dependent myeloid differentiation primary response protein 88 (MyD88) that activates nuclear factor-κB (NF-κB). As a result, the production of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factorα (TNFα) occurs, which controls viral pathogens (Haase et al., 2008; Brieger et al., 2013; Gupta et al., 2022). In another study, El-Megharbel et al. (2021) synthesized zinc oxide nanoparticles, and the antiviral activity of synthesized particles was checked against SARS-CoV-2. The zinc oxide nanoparticles were used as a spray for surface coating, and results revealed that in an *in vitro* study, at a very low concentration of IC_{50} = 526 ng/ml, the antiviral activity was $CC_{50}/IC_{50} \leq 1$. The authors recommended these nanoparticles' spray as a good disinfectant, but it has some cytotoxic effects, CC_{50} = 292.2 ng/ml to the host cell (El-Megharbel et al., 2021). SARS-CoV-2 has become a matter of concern for scientists during the last 2 years. Many researchers have focused on anti-SARS-CoV-2 activity using different metals. Attia et al. 2021 synthesized zinc oxide nanoparticles using hesperidin (isolated from the orange peel) and checked it against the hepatitis A virus (an example of RNA virus). The results show that the synthesized nanoparticles possess 58.83% activity with minimum cytotoxic concentration.

In addition to metals, metal slats and their nanoparticles reported in the literature are compiled in Table 1. Copper, silver, and zinc are the most common types of metals. Iron also has shown activity, but reports on iron are limited. Copper-based nanoparticles in small doses show faster kinetics in virus deactivation.

3.2 Fullerene and its derivatives

Fullerenes are allotropes of carbon with unique properties. They are used in the preparation of advanced functional materials as a scaffold because of their good biological properties. Fullerenes have gained the attention of researchers due to their water solubility and cyclopentadienyl pattern. They are widely used in self-assembled nanostructures, siRNA AND gene delivery, and DNA binding (Maeda-Mamiya et al., 2010; Minami et al., 2014; Nitta et al., 2015; Castro et al., 2017; Kraevaya et al., 2021; Shi et al., 2021). Dating back to 1993, the antiviral activity of fullerene has been known against HIV. Fullerenes and derivatives showed significant antiviral effects against herpes simplex virus (HSV), Ebola virus, cytomegalovirus (CMV), and HIV influenza virus (Friedman et al., 1993; Mashino et al., 2005; Fedorova et al., 2012; Tollas et al., 2014; Muñoz et al., 2016). Fullerene derivatives are mostly water soluble, broadly characterized into six main types: 1) glycofullerene derivatives; 2) carboxyl derivatives; 3) amino acid, peptide, and primary amine derivatives; 4) hydroxyl derivatives; 5) fullerene complexes; and 6) piperazine and pyrrolidine derivatives (Xu et al., 2022). Fullerene blocks encoded enzymes and fit well on the protease of HIV. In order to block the HIV enzyme, the diamido diacid diphenyl fulleroid was designed first. Then, other groups were synthesized to inhibit the HIV enzyme, DNA polymerase, and HIV-1RT (Sijbesma et al., 1993; Nacsa et al., 1997; Castro et al., 2016; Innocenzi and Stagi, 2020; Xu et al., 2022). Coating of fullerene derivatives on the surface can prevent the possible attachment of SARS-CoV-2 because it produces lipid peroxidation on the lipid layer of SARS-CoV-2 and creates a hydrophobic surface, which minimizes the contact between the surface and the virus due to air entrapped between topographies. These textured surfaces possess anti-biofouling properties (Siddiquie et al., 2020). A visual diagram for probable deactivation is shown in Figure 2. Kraevaya et al. (2021) used fullerene derivative $C_{60}Ar_5C_l$, with thiophenes, and the antiviral

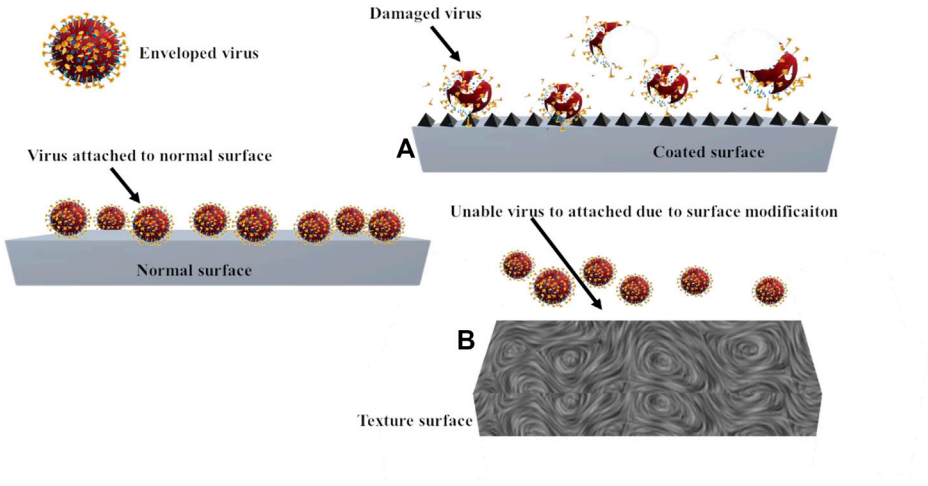


FIGURE 2
Texturing of the surface with fullerene derivatives for virus inactivation. By surface modification **(A)**. Fullerene-textured surface **(B)**.

activity against influenza A (rimantadine-resistant) Puerto Rico/8/34 H1N1 was evaluated. The synthesized compound K10, with five residues of thien-2-yl acetic acid and hydrocinnamic acid, showed very low cytotoxicity CC_{50} ~ 69 μM and good antiviral activity EC_{50} ~ 3 μM. In another study, Troshina et al. (2007) synthesized fullerene carboxylic acid derivatives (chlorofullerene C60Cl6) at a pH < 7.5. The water solubility of the reported derivative was about 50–100 mg/ml, with low cytotoxicity greater than 52 μM, and the antiviral activity *in vitro* and *in vivo* against HIV-1 was reported with IC50 of 1.20 ± 0.4 μM.

3.3 Graphene and its derivatives

Graphene has been used in the inactivation of different kinds of viruses and microbes due to its higher surface area, electric conductivity, movement, and mechanical and piezoelectric characteristics (Yang et al., 2014; Zhang et al., 2015; Quan et al., 2017; Zhang et al., 2019; Lu et al., 2020). At first, the viral envelope is damaged when it interacts with graphene oxide, which leads to the production of ROS and virus deactivation (Gurunathan et al., 2012; Díez-Pascual, 2020; Ayub et al., 2021). Sametband et al. (2014) reported inhibitory properties of graphene oxide and sulfonated graphene oxide against HSV-1. Graphene oxide and sulfonated graphene oxide possess negatively charged particles like in the heparan sulfate cell receptor. As a result, both moieties compete to bind with HSV-1. The graphene blocks the binding sites, acting as an inhibitory agent (La Rosa et al., 2020). In terms of water safety, viral transmission possesses a substantial threat. Viruses, such as rotavirus, adenovirus, and norovirus, can pollute water, becoming the major cause of gastroenteritis (non-bacterial) (Bridge et al., 2010; De Graaf et al., 2016; Xue et al., 2019). These viruses are highly resistant to stressors possessed by the environment and risk of mortality in less immune individuals, young and elderly ones (de Roda Husman and Bartram, 2007; Espinosa et al., 2008). Zhou et al. (2022) developed a reduced graphene oxide composite nanoparticle functionalized with CTAB (cetyltrimethylammonium bromide) to absorb SARS-CoV-2 and rotavirus, adenovirus, and norovirus (human enteric viruses). The functionalized with CTAB, RGO-Fe_3O_4 have shown good absorption toward SARS-CoV-2, HAdV, HuNoV, and HRV with a maximum absorption capacity of 6.92×10^6, 2.21×10^7, 7.01×10^7, and 3.55×10^7 genome copies mg^{-1}. The study revealed that viruses are trapped on the surface of synthesized composite by intrinsic absorption and electrostatic interaction between composite and virus. The synthesized composite can absorb the abovementioned viruses from rivers, coasts, and tap water. Unal et al., 2021 worked on graphene oxide nanosheets to inhibit the infectivity of SARS-CoV-2 cell receptors and surface protein. The study revealed that graphene oxide sheets work as a nanomaterial that can interact with SARS-CoV-2 specific receptors and surface proteins to inhibit viral infection. To understand the interaction between graphene oxide and the spike of the virus, spike-ACE2 complex, ACE2 cell receptor, and molecular docking experiments have been conducted. The results of molecular docking revealed that graphene oxide sheets interact with three types of structure present in SARS-CoV-2: closed state -6VXX is a type of spike protein of 2019-nCoV (novel coronavirus) or open state -6VYB (viral spike), open state -6VYB or closed state -6VXX (ACE2-bound spike complex), and 1R42 (ACE2). Graphene oxide showed good binding

affinities toward all three surface structures 6VXX, 6M0J, and 6VYB. Graphene oxide shows more binding affinity toward ACE2 or spike compared to 6M0J. The obtained data shows that graphene oxide sheets tend to disturb the infectivity of SARS-CoV-2 even if any mutations are present on the viral spike. Deokar et al. (2017) demonstrated the synthesis of magnetic particles for the destruction of HSV-1. The author synthesized sulfonated nanoparticles with magnetic nature functionalized on reduced graphene oxide with a smaller size of approximately 5–25 nm. The synthesized graphene-based functionalized material can inactivate the HSV-1 virus up to 99.99% within 7 min. Magnetic functionalized graphene oxide captures the virus using an external magnet, which enhances the photothermal treatment. Donskyi et al. (2021) investigatedsulfate/alkyl functionalities on graphene sheets for the inactivation of SARS-CoV-2. Different lengths of alkyl chain have been synthesized with graphene oxide to determine the inhibition of feline coronavirus and SARS-CoV-2. It has been found that an alkyl chain greater than C9 can disturb coronavirus replication by disturbing its envelope. The antiviral activity against SARS-CoV-2 of the synthesized graphene oxide with dual functionalities with aliphatic chains and PGS (polyglycerol sulfate) showed IC_{50} (30 μg ml^{-1}). The lower surface energy of certain materials and roughness at the nanoscale can play a great role in increasing the antiviral properties of materials, especially in the case of treated fabric or PPE. It can increase antiviral properties and superhydrophobicity. Galante et al. (2022) reported an antiviral fabric coating functionalized with coal-derived nano-graphene oxide. The graphene oxide was functionalized with octadecyl amine to increase water-repellent properties. The functionalized material is used to coat PET (polyethylene terephthalate) fabric. Furthermore, the polydimethylsiloxane (PDMS) layer was coated on the fabric to increase durability and repellency from human saliva and other fluids. The functionalized graphene oxide fabric was tested against five different types of viruses. The results revealed that the developed fabric had reduced viral titers of CoV reduction by 99.6% (2.4 logs), HAdV5 reduction by 98.6% (1.8 logs), and HSV-1 reduction by 99.4% (2.2 logs). The functionalized fabric repels droplets of human saliva and other fluids. Even after washing with bleach or mechanical abrasion, the fabric can reduce viral quantity up to 1–2 logs for a wide range of enveloped and unenveloped viruses.

3.4 Photocatalytic materials

We have discussed various surface coating antiviral materials and their antiviral activities to effectively control the viral infectious disease in hospitals or at home. Photocatalytic surfaces have gained more attention from researchers due to their constant inactivation, oxidizing, and destruction of microbes under ambient indoor environments (Reid et al., 2018; Rtimi et al., 2019; Miyauchi et al., 2020; Hamdi et al., 2021; Micochova et al., 2021; Prakash et al., 2021). Various photocatalysts have been used as antiviral and antibacterial materials, among which TiO_2 is well known due to its viral and bacterial disinfection properties, as well as degradability for pollutants (Hajkova et al., 2007; Park et al., 2014; Akhtar et al., 2019; Moongraksathum et al., 2019). Exposing TiO_2 to UV-A light, the ambient water and oxygen decompose on the surface of TiO_2 in superoxide anions and hydroxide radicals. These substances are responsible for the decomposition of microbial and organic matter, which results in adverse effects, adducent microbial cells because of the peroxidation of lipid membrane (phospholipids present in the membrane) (Hajkova et al., 2007). Hajkova et al. (2007) also reported the photocatalytic antibacterial and antiviral effects of TiO_2 films, which deactivate viruses up to 100% antiviral within 6 h of illumination. A few reports have been conducted on photoactive virucidal surfaces. For example, TiO_2 photocatalysts were prepared and coated on aluminum and stainless surfaces. Ultrathin TiO_2 coatings were obtained by wash-coating and screen-printing techniques. The later provides films of excellent adhesion that could tolerate washing under a water jet. The catalyst also exhibited excellent bactericidal, fungicidal, and virucidal activities against a wide variety of Gram-positive and Gram-negative bacteria, fungal spores, and T2 bacterial phage (Yao and Lun Yeung, 2011). Bactericidal and virucidal covering materials and methods for making the covering material are patented, where photocatalytic material is incorporated into thermoplastics. The sheets can be used in hospital settings to protect the furniture (Corsi et al., 2015). The self-disinfection properties of metal oxide are one of the best tools to stop the spreading of viral contamination from the infected person to an inanimate surface and from an inanimate surface to the healthy one. TiO_2 is useful for photocatalysis because it has antiviral, antibacterial, and antibacterial characteristics. Excellent virus inactivation under UV light can be achieved by depositing a thin coating of TiO_2 nanoparticles on a glass surface or another inert surface. TiO_2 possesses good qualities regarding photocatalytic activities and antibacterial and antiviral properties. A thin layer of TiO_2 nanoparticles deposited on the surface of glass or another inanimate surface can give excellent virus deactivation under UV light. In contrast, TiO_2 dopped in cobalt nanoparticles was reported as a cost-effective good detection option for SARS-CoV-2 infection using electrochemical, biosensor in saliva, or nasal secretions based on coronavirus spike protein sensing. The larger surface area and catalytical and antiviral properties have made TiO_2 nanoparticles an excellent candidate. Photoactive TiO_2 hydrothermally grown can form reactive oxygen species, particularly hydroxyl radicals ˙OH, under the low light of 0.4 mW cm^2 and wavelength of 375 nm, giving good pathogen interaction including SARS-CoV-2 (Kumar et al., 2020; Kumar et al., 2022). Thin films of TiO_2 on

glass for self-cleaning have been recently reported as a chapter in the book by Sirichantra (2022). The study recommends that such antiviral glasses would be useful in ambulances (Sirichantra, 2022).

4 Other virucidal nanomaterials

Carbon-based nanostructured photocatalysts have attracted attention, especially in the photocatalytic disinfection of microorganisms. With the appropriate electronic band gap structure and distinctive features of high thermal and chemical stability, a metal-free 2D polymeric stacked structure g-C_3N_4 (graphite carbon nitride) is a promising photocatalytic material for energy and environmental applications. It also has great potential for the inactivation and degradation of pathogens. The disinfection of microorganisms is mainly attributed to the formation of reactive oxidative species (ROS). The surface modification of g-C_3N_4 can significantly improve photocatalytic disinfection efficacy (Dang and Tarabara, 2019).

5 Deposition methods of nanoparticles on a fabric substrate

In the textile industry, the application of nanotechnology has good potential. The properties of fabrics are generally improved due to nanomaterials or the new abilities added to textiles (Karst and Yang, 2006; Motakef Kazemi and Sandalnia, 2020).

Cotton fabrics provide a perfect environment for bacteria and fungi growth because the moisture, temperature, and nutrition (skin dead cells, stains, sweat, and other skin emissions) on the surface of the fabric match their development and reproduction requirements. Microbes that live on the fiber surface can cause unpleasant smells, dye degradation, allergic reactions, textile deterioration, and even health issues. As a result, making antimicrobial textiles has attracted the attention of a broad array of researchers, and the market for antimicrobial textiles is quickly growing. Silver, triclosan, biguanide derivatives, N-halamines, peroxyacids, quaternary ammonium salts, and synthetic colors are examples of antibiotic compounds (Zhang et al., 2016). Nanostructured materials based on metal nanoparticles have been intensively explored for diverse applications due to their appealing physical, chemical, and catalytic properties (Wiener et al., 2013). The molecular alteration of textile fabric to produce innovative materials that are several times more effective than untreated fabric is continually progressing. These materials focus on modifying the fabric surface for enhanced antibacterial properties, soil-resistance, water-repellency, antistatic, anti-infrared, and flame-retardant qualities of traditional textiles. Fabrics possess a huge surface area and pore volume. Thereby, nanoparticles quickly adsorb and can inactivate the bacteria and other microbes (Khan et al., 2020). The nanoparticles and structures are almost identical in size to biological molecules, which makes them an interesting candidate for biological study *in vivo* and *in vitro* (Nienhaus et al., 2020). They would have a wide range of uses in synthetic textiles; biomedical, surgical, and water treatment; equipment; food processing; and packaging (Alagarasan et al., 2021).

In natural and synthetic textiles, nanoparticle-based coatings are quite common and come in different compounds. Silver nanoparticles (AgNPs) have a high level of toxicity against a wide range of bacteria but have a low level of harmfulness to human cells and durable stability. Nanomaterials made of silver (Ag) are well known for their self-cleaning and antimicrobial properties (Goharshadi et al., 2022). The antiviral activity of AgNPs (size less than 10 nm) has also been observed against COVID-19. Moreover, silver, other metals, and metal oxide nanoparticles, such as gold, zinc, tin, titanium, and copper, are used in natural and synthetic fabrics. As explained previously, three basic mechanisms can be linked to the antibacterial activity of functionalized CuO NPs on textile materials against Gram-positive and Gram-negative bacteria: copper ion release, direct interaction of CuO NPs with bacteria, and the formation of reactive oxygen species (Gulati et al., 2021). Many surfaces are frequently touched and soiled with viruses (Figure 3), which require special attention for disinfection. Coating such surfaces with antivirals can significantly reduce the transmission of viruses and other microbes. Not much work is done in this direction. However, it is a very crucial area. In the following sections, we will focus on methods reported in the literature to deposit antiviral compounds on high-touch surfaces and fabrics.

Various methods are reported in the literature to deposit antivirals onto different surfaces. Table 2 shows the different methods besides the adopted methods, dip-coating, and sonication.

5.1 Dip-coating process

The dip-coating method is a simple and efficient technique. This method is commonly used in industries to deposit any substrate such as metallic, polymer films, and ceramic and fibrous materials. The deposition process can be defined as the coating of aqueous-based liquid phase solutions onto the surface of any substrate, and then the wet coating object is dried at room temperature (Tang and Yan, 2017). Furthermore, Lu et al. focused on endowed silk to introduce the antibacterial activity and UV shielding properties by depositing CeO_2 nanoparticles on the surface of silk through the dip-coating method. Thermal stability properties are evaluated using thermogravimetric analysis and its derivatives. The successfully coated CeO_2 nanoparticles on silk, the ability of UV-protection, and the antibacterial property were confirmed in UV–Vis diffuse reflectance spectroscopy and colony-forming

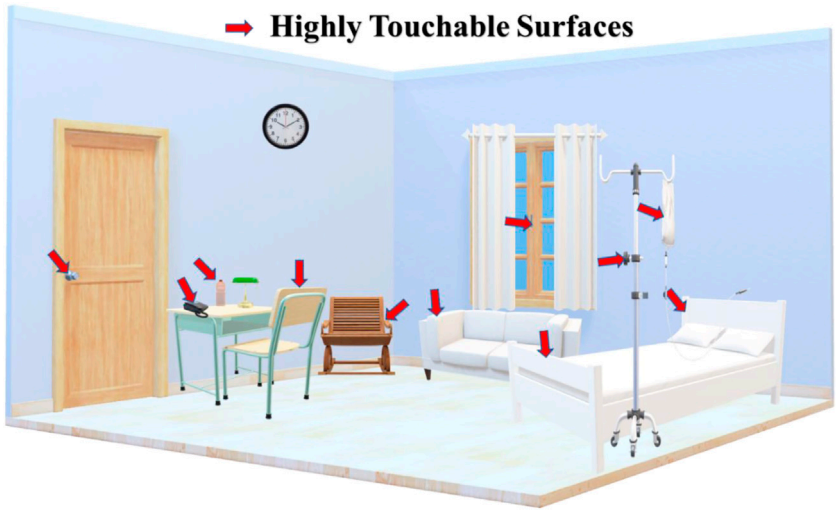


FIGURE 3
High touch surfaces in hospital settings.

capability test, indicating that CeO_2 nanoparticles are successfully coated on silk and are the best-modified material for applications of UV-protection and antibacterial applications (Bhattacharjee et al., 2020). Another material was prepared using reduced graphene oxide (RGO) coated-copper (Cu)/silver (Ag) nanoparticles on carbon cloth *via* an easy dip-coating method and using a coupling agent (3-glycidyloxypropyl trimethoxy silane). The RGO and Cu/Ag composites were simultaneously coated on cotton cloth samples, which showed improved hydrophobicity compared to pure cotton. The surface resistance of cotton-RGO-Cu is 6.42 KΩ/sq, with a high UV protection factor (46.45). Furthermore, after 20 wash cycles, the resistance of cotton-RGO-Cu is 16.70 KΩ/sq. The cotton-RGO-Cu fabric properties include enhanced hydrophobicity, low surface resistance, better UV protection, and thermal stability (Bhattacharjee et al., 2020). In another work, the synthesized zinc oxide nanoparticles were coated on cotton fabrics. The zinc acetate solution in various concentrations with monoethanolamine with 2-methoxy-ethanol as solvent was used to obtain nanoparticles after an aging time of 24 h. The different concentrations of nanoparticles were coated on cotton fabric *via* the dip-coating method. The 3 M concentrations of ZnO were well-dispersed on the fabric shown *via* SEM images. The UV absorbance increase with the increase in the concentration. The 2 M concentration range was found to have the maximum antibacterial and antifungal activity (Roy et al., 2020). This report discusses the low-cost method of coating nylon fabric with an antimicrobial application.

A blend of chitosan and Ag nanoparticles coating on fabric *via* easy dipping with different concentrations of materials is reported. The synthesized silver nanoparticles using the Lee–Meisel method showed the surface plasmon resonance band at 410 nm corresponding to a stable average nanoparticle diameter of 25 nm. The prepared fabric controlled higher bactericidal activity (Gram-positive *Staphylococcus aureus*) compared to (Gram-negative *Pseudomonas aeruginosa*) bacteria. The result showed 20% reduction in *S. aureus* and 60% reduction in *P. aeruginosa* CFU, when each coating combination is exposed. The antimicrobial effect was decreased after several washes, indicating that after washing the fabric, the coated material becomes unstable and can be easily removed. However, for the purpose of single-use face masks, new-coated material is favorable (Roy et al., 2020).

In a report, SiO_2 nanoparticles are coated on cashmere fabric *via* the dip-coating method using acetic acid to produce a highly superhydrophobic surface. Cashmere fabric had a superhydrophobic characteristic with a contact angle greater than 150°C, according to contact angle measurements. The coated material on the fabric does not affect the cashmere fabric. The colorfastness and pilling grade of the surface-coated cashmere were improved, so the coating method had no bad impact on the cashmere fabrics' quality (Botelho et al., 2021).

In this report, graphene oxide (GO) and cuprous oxide (Cu_2O) were produced and uniformly coated on polyethylene terephthalate (PET) fabric with excellent adhesion using dopamine.

The findings show that GO acts as a Cu_2O nucleation site while avoiding agglomeration, and during Cu_2O reduction, it is rehabilitated to RGO. The nanoparticles coated on PET fabric show great antibacterial activity against *S. aureus* and

TABLE 2 Deposition methods for preparing antiviral surfaces.

Supporting surface	Antiviral coating material	Coating technology	Virus	Efficiency (%)	Stabilizing time (h)	Ref.
Fabric/mask/nylon fibers	CuS	Thread modification	SARS-CoV-2	80	5–10 min	Hewawaduge et al. (2021)
Variable surfaces	Laser-induced graphene (LIG)	CO_2 laser	HCoV-OC43	97.5	15 min	Huang et al. (2021a)
	Hydrophobic LIG (HLIG)		HCoV-229E	95%		
Glass slide	N,N-Dodecyl,methyl-polyethylenimines (PEIs)	Coating paints	Influenza virus	100 (4-log reduction in the viral titer)	1–2 min	Haldar et al. (2006)
Stainless steel coupons	Quaternary ammonium polymer coating (antimicrobial coating)	Electrostatic sprayer	Human coronavirus 229E	Reduction log > 3.99	120 min	Ikner et al. (2020)
Glass slides	Polycation N,N-dodecyl, methyl-polyethylenimine	Coating (painting)	*Staphylococcus aureus* and *Escherichia coli*, influenza virus A/Victoria/3/75 (H3N2) and A/WSN/33 (H1N1)	100	2 h	Haldar et al. (2007)
Glass coverslips	Silica nanoparticles (SNPs) coated with a didodecyldimethylammonium bromide (DDAB) surfactant, didodecyldimethylammonium bromide (DDAB)	Coating	Bacteria, molds, yeast, and influenza A/PR/8/34 (H1N1)	100	–	Botequim et al. (2012)
Nanocomposite thin film	Cu–plasma-polymerized fluorocarbon (PPFC)	Sputtering method	Influenza virus A	96.9	30 min	Kim et al. (2021)
Polypropylene KF94 mask	Copper thin film (cuprous oxide)	Vacuum coating	SARS CoV-2	75	30 min	Jung et al. (2021)
Glass	Copper–graphene (Cu–Gr) nanocomposite	Dip-coating method	Influenza A virus, strain A/H1N1/WSN/1933	64	30 min	Jana et al. (2021)
Aluminum 6,063 alloy sheets	Fabricated nanoscale features on aluminum alloy surfaces	Wet etching technique	*Pseudomonas aeruginosa*, *Staphylococcus* Respiratory syncytial virus (RSV) and rhinovirus (RV)	87–92	24 h	Hasan et al. (2020)
	Sodium hydroxide-based wet-etching process			3–4 $\log_{10}$ reduction		
fiber mat	Silver nitrate and silver nanoparticles	Coating with electrospinning technique	Murine norovirus (MNV-1 strain)	1.42 and 0.14 log TCID50/ml	24 h	Castro-Mayorga et al. (2017)
Nano-particles	Sulfonated magnetic nanoparticles functionalized with reduced graphene oxide (SMRGO)	Photothermal treatment	Herpes simplex virus type 1 (HSV-1)	99.9	10 min	Deokar et al. (2017)
Painting or spraying	Quaternary benzophenone-based ester and quaternary benzophenone-based amide	Surface coatings	Influenza virus	100	30 min	Ghosh et al. (2020)
Ceramics	La2Mo2O9 ceramics	Complex polymerization	Qβ and bacteriophage Φ6	99.9%	6 h	Matsumoto et al. (2019)

E. coli, more than 99.99%. However, after 40 washes of modified PET fabric, the antibacterial rates were 90% for *S. aureus* and 88% for *E. coli*. The decrease resistivity to 7.16 × 108 Ω cm from 2.64 × 1,015 Ω cm of the modified PET fabric range. Additionally, the UV protection was improved from 45 to 460, far exceeding the excellent rating (50). Overall, in the textile field, different pathways are opened for different applications (Hu et al., 2022).

5.2 Sonochemical coating process

The sonochemical method is one of the versatile methods for coating purposes. In 1975, a textile finishing process was first reported by ultrasound exposure through deep ultrasonic irradiation and cross-linking resins such as urea-formaldehyde. Harifi and Montazer (2015) published an outstanding review about textile sonoprocessing, describing the surface finishing of fabrics using various metals, metal oxides, and combinations and achievements. Many studies have been conducted using the sonochemical method to deposit nanoparticles [copper oxide (CuO), zinc oxide (ZnO), titanium dioxide (TiO_2), magnesium oxide (MgO), silver (Ag), copper (Cu), Ag/TiO_2, Zn/CuO, etc.]. Inorganic particles are used to functionalize textiles (Perelshtein et al., 2015; Perkas et al., 2018; Patil et al., 2020). The large surface area is a beneficial property of metal nano-oxide, which is suitable for coating textile fabrics. The market of antimicrobial NPs of metal oxide is a better substitute for quaternary ammonium salts, triclosan, and other toxic compounds. The main advantage of sonochemically coated textiles is that after 65 washing cycles, the coated fabric has good antibacterial efficiency. Furthermore, color and biocide functions are added to textiles through the sonochemical coating method. The composite ZnO/chitosan nanoparticles coated on textile through the sonochemical method were used to increase the antibacterial activity. The ZnO and chitosan are coated on fabric by a one-step deposition process and form hybrid antimicrobial layers. The antibacterial properties of the textiles were improved, as well as their biocompatibility. The sonochemical coating technique for 30 min using a dispersion of 2 mM ZnO nanoparticle provided an antibacterial effect for two pathologically important bacterium species. The responsivity against *Staphylococcus aureus* and *Escherichia coli* was higher for chitosan and ZnO deposited with the same quantity of only ZnO. The antibacterial robustness effects were increased by 21% for *S. aureus* and 40% for *E. coli* due to the presence of biopolymers. The coated fabric was stable for many washing cycles under hospital laundering conditions. (Petkova et al., 2014). Recently, Kwiczak-Yigitbas et al. (2020) reported an ultrasonication method for the synthesized environmentally friendly cellulose fabrics comprising silver or gold nanoparticles. The authors reported cellulose mechanochemistry in terms of breaking glycosidic bonds and producing mechano-radicals. The reduced metals can be stabilized by the cellulose chains on nanoparticles, and these mechano-radicals can decrease Au3þ and Agþ ions in the solution. Silver nanoparticle–fabric composites with antibacterial properties and the catalytical composite of active gold nanoparticle–fabric with metal ion reduction properties yield up to 14% using this approach. The cloth is sonicated in aqueous solutions without the use of harmful reducing and stabilizing agents. The availability of coated fabric for medical textile applications is rapid and environmentally friendly (Kwiczak-Yigõitbaşı et al., 2020).

5.3 Pad-dry-cure process

Different deposition processes can be used for textile fabrics utilizing modified chitosan created using metal and metal oxide nanoparticles to generate novel function materials. Using the precipitation process, some researchers have synthesized chitosan–ZnO nanocomposite. Bio-nanocomposite materials were coated on fabric *via* pad-dry-cure (Beninate et al., 1968) and sol-gel (Sogorkova et al., 2018) methods to increase their washing elasticity. In some instances, the washing robustness of the coatings is improved using (3-glycidyloxypropyl) trimethoxysilane. The performances of antibacterial and UV protection were tested after the bio-nanocomposite coated on fabrics. The prepared chitosan–ZnO–TiO_2 nanocomposite was also used to observe the variations in UV properties. Binary chitosan–ZnO composite shows good results for antibacterial and UV protection. The treated cotton fabrics improved the impacts of multiple washing cycles compared to simple chitosan-treated materials. By using a ternary coating composite, the UV protection factor is elevated to an excellent level.

Coating multifunctional technical cotton textiles using smart biomaterials is a revolutionary approach to multifunctional cotton textile design. Two distinct (NC1, NC2) ternary nanocomposites, containing (ammonium-salicylidene) chitosan Schiff base (ASCSB), TiO_2, and ZnO nanoparticles, were successfully synthesized *in situ* and coated to cotton fibers using the simple pad-dry-cure procedure to impart antibacterial and UV protection properties. NC1 has much TiO_2, whereas NC2 has much ZnO. Spectral, microscopic, and thermal approaches were used to evaluate the physicochemical and graphic features of the novel nanocomposites. NC1 exhibited a more homogeneous distribution, higher depositing density and smaller mean nanoparticle size (48 nm) when compared to NC2 (56 nm). NC2-treated fabrics, on the contrary, had a greater nanoparticle depositing than NC1-treated fabrics. The treated cotton fibers had robust and long-lasting antimicrobial effects against *S. aureus*, *E. coli*, and *Candida albicans* pathogens, with NC2-treated textiles performing better than NC1-treated textiles. The NC2-remediated cotton fabrics demonstrated a higher UV protection factor (UPF) value (53) as compared to NC1-coated fabrics (35), revealing that rich-ZnO nanocomposite provided higher UV protection to cotton fabrics than the rich-TiO_2 nanocomposite (Refaee et al., 2022).

Carboxymethyl chitosan and Ag/TiO_2 composite nanoparticles were coated on fabrics for long-term antibacterial and UV protection (Xu et al., 2021). The Ag/TiO_2 colloid solution was stabilized with carboxymethyl chitosan, and the carboxymethyl chitosan and Ag/TiO2 composite nanoparticles were subsequently coated on cotton utilizing the pad-dry-cure process. The excellent properties of modified fabric with the bacterial reduction of 99.5% and UV protection factor of 79.0, respectively.

Moreover, the coated fabric properties showed no change up to 50 washing cycles.

5.4 Spray-coating

The spray-coating method can be used to create superamphiphobic polymer coatings quickly and efficiently. Several surfaces have been examined as substrates for this coating technique, including paper, glass, cotton, aluminum, and copper (Steele et al., 2009; Wang et al., 2014). To create the desired coatings, different polymer-based fluorobinders (with low surface energy) and nanoparticles, such as silica and ZnO nanoparticles (denoted as SNs and ZNs, respectively, giving microscale and nanoscale roughness), are commonly mixed as building blocks and spray-coated onto a substrate. Furthermore, fluorinated SNs (FSNs) were employed to create coatings with increased durability with clearness (Wu et al., 2016). Sasaki et al. used double-walled carbon nanotubes (DWCNTs) combined with gold nanoparticles (AuNPs) *via* a simple and low-cost spray-coating approach to exhibit flexible conductive fabric (Yotprayoonsak et al., 2022). The rising need for surgical masks, as well as their disposal, has resulted in considerable financial and environmental expenses since the advent of the COVID-19 pandemic. The researchers used a dual-channel spray-assisted nanocoating hybrid of shellac/copper nanoparticles (CuNPs) to increase the hydrophobicity of a non-woven surgical mask and repel aqueous droplets. The resultant surface exhibits excellent photoactivity for antimicrobial action (combined photocatalytic and photothermal capabilities), allowing the masks to be reused and self-sterilized. This photoactive antiviral mask (PAM) immediately reached a temperature of >70°C when exposed to sunlight. The masks became self-cleaning and reusable. This PAM architecture can protect against viral infection transmission (Kumar et al., 2020). Copper is a common substance that has antibacterial and antiviral properties. The antiviral activity of copper nanoparticles (CuNPs) was investigated against SARS-CoV-2 as a surface coating agent. The diameter range of 254 nm of CuNPs was formed using copper sulfate as a source and was primarily made up of CO. Combined CuNPs, and resin-based paint sprayed on the stainless-steel surface remains (CuNP/paint). After 30 min of exposure, SARS-CoV-2 lost 97.8% of its infectivity on the CuNP/paint-coated surface, and in the following 1 h, it lost more than 99.995% of its infectivity. The inactivation rate on the paint alone-coated and uncoated surfaces was roughly 36-fold faster. Although more studies are needed to explain the inactivation processes, the CuNP/paint-coated surface displayed strong inactivation of SARS-CoV-2 infectivity. This coating material is expected to be useful in public hospitals and other frequently handled places (Purniawan et al., 2022).

Using a one-step spray-coating method, we describe a straightforward and universal strategy for optimal coupling superhydrophobic and antibacterial properties on diverse textiles. This is accomplished by adhering fluorinated mesoporous silica nanoparticles (F-MSNs) and quaternary ammonium-functionalized MSNs (Q-MSNs) to various textile surfaces using PDMS as a binder. CAs of 152 and SAs of 2 indicate that the resultant double-nanoparticle arranged coatings on textiles (F/Q-MSNs coatings) have significant antibacterial activity against *E. coli* and *S. aureus* due to the "repel-and-kill" synergic effect and excellent waterproof and bacterial shielding function. In addition, F/Q-MSN coatings are resistant to sandpaper abrasion, washing, and strong acid/alkaline. Furthermore, the F/Q-MSN coating can maintain the textile's original application characteristics, such as breathability and deformability. As a result, F/Q-MSN has developed antibacterial textile coatings that are very practicable, adaptable, and universal, with potential and diversified applications in various fields (Ye et al., 2021).

Many academic and industry researchers have attempted to produce fabrics with superhydrophobic properties inspired by various environmental life forms' excessive wettability, such as lotus leaves' high-water repellency. A novel wettability switching mechanism has recently sparked a surge in demand for advanced coatings, even though their production remains difficult and expensive. Cotton fabrics with irregular wettability (one face with natural superhydrophobicity, the other face with natural superhydrophilicity) were made by spraying a biocompatible commercial material mixture, hydrophobic SiO_2 nanoparticles, and ethyl-alphacyanoacrylate superglue onto the fabric in one step. The method involves adjusting the distance between the sprayer and the fabric to make one side naturally superhydrophilic and the other superhydrophobic, thus managing the absorption of the fabric coatings. As a result of its great mechanical durability, the superhydrophobic side has a surface with a water contact angle of 154°C and a sliding angle of 16°C meet the standards for self-cleaning capabilities. Due to the fabric's intrinsic superhydrophilic feature, the reverse side had a strong water absorption ability. Furthermore, the superhydrophilic side of the created cotton materials consumed blood absorption and clotting characteristics. On the contrary, the superhydrophobic side prohibited water and blood infiltration sacrificing the cotton's intrinsic breathability. These features could be important in the development of multifunctional materials for medical purposes (Sasaki et al., 2016)*.

5.5 Magnetron sputtering

Cathodic magnetron spraying has become the process of choice for the deposition of a wide range of coatings of industrial

importance. Examples include hard and wear-resistant, low friction, corrosion-resistant, decorative coatings, and coatings with specific optical or electrical properties. In the primary sputtering process, a target plate (or cathode) is bombarded by energy-sensitive ions produced in the glow-emitting plasma located in front of the target. The bombardment process causes the removal of the target atoms (i.e., sputtering), which can then condense on the substrate as a thin film (Kelly and Arnell, 2000). The deposition rate and coating thickness are influenced by the ionization and collision rates of argon (a gas used to generate plasma) on the target material in the sputtering system. A magnetic field can be produced by placing the magnets underneath the target. These secondary electrons trap through the magnetic field that the target emits into the discharge and raises the ionization of the gas, which increases the rate of collisions between the Ar^+ and the target material. In changing the target material's chemical makeup and the operational conditions, the stoichiometry of the film can be easily changed. Introducing N_2, C_2H_2, and O_2 gases together with the Ar gas, nitride, carbide, and oxide coatings may be easily created (Golosov, 2017). The most common substrates for the deposition of films using MS techniques are metals or glass. However, in recent years, the application of MS for the deposition of metallic and oxide coatings on textiles has become more widespread (Liu et al., 2017; Antunes et al., 2021; Huang et al., 2021b). Most of the coated films are tested against antibacterial properties (Kim et al., 2018; Markowska-Szczupak et al., 2021; Woranan thakij et al., 2022), whereas limited literature is available on MS-coated films for antiviral assessment.

Table 3 lists various MS modes used to create coatings on various substrates to develop antiviral surfaces. A highly effective antiviral surface coating, a highly imperfect, amorphous TiOx (x 1.2), was created. The antiviral activity was noticeably stronger, comparing this amorphous shape to the more often studied crystalline anatase phase. It was discovered that the covering was remarkably clear. This film was created at room temperature without the need for any post-deposition thermal treatment using radio frequency (RF) magnetron sputtering, one of the well-known industrial-scale deposition methods. The potential for commercialization of this antiviral surface coating is enormous due to its affordability and environmental friendliness (Mittireddi et al., 2021). Applying a reliable method based on Cu magnetron sputter-deposition onto electrospun polymer nanofibers, the Cu-coated PCL (polycaprolactone) nanofibrous mats were effectively created (Manakhov et al., 2021). For the first time, molecular dynamics simulation was used to describe the large-scale irradiation of PCL films, allowing for the prediction of ion penetration depth and fine-tuning of deposition conditions. Because of the quick release of Cu_2^+ ions (concentration up to 3.4 μg/ml), acceptable biocompatibility, and antibacterial activity against *E. coli* and *S. aureus*, the copper-coated PCL nanofibers demonstrated that they were effective. Thus, when used for wound healing, they might show an intriguing synergistic effect. Because copper ions are required for immune system cells to act in a bacteriostatic way, the quick discharge of copper ions simultaneously kills germs and activates immune cells, stimulating regeneration.

The potential of plasma techniques was reviewed by Ma et al. (2021), concluding that "impressive recent advances in plasma functionalization of polymer surfaces suggest that plasma-assisted surface functionalization approaches are promising for the production of antiviral polymers with targeted antiviral applications ranging from *in vitro* prevention to *in vivo* therapy."

6 Antimicrobial textiles and fabrics for protective applications

The textile industry is flourishing, and there is an urgent need to develop environmentally friendly and effective antimicrobial fabrics (Mallakpour et al., 2021). Healthcare services are crowded with patients having different diseases, where harmful microorganisms are more prevalent than in other places. Additionally, employees of healthcare facilities are at high risk for viruses. Compared to the general population, healthcare employees are more at risk; therefore, the use of antiviral fabrics is of utmost importance in healthcare centers.

During the COVID-19 pandemic, around 210 countries and all regions were affected, mostly *via* respiratory droplets. The doctors, patients, and the public used disposable surgical masks in high-risk areas. Masks played an important role during the COVID-19 pandemic, the spread of droplets was blocked, and the health of people was safeguarded. Recently, many researchers worked on antivirus masks (Tuñón-Molina et al., 2021). Borkow et al. reported a copper intermediate layer with an antiviral mask, containing four layers. The A and B layers consist of the outer and inner layers: (A) spunbonded polyamide fabric containing 2.2% weight (w/w) copper particles; (B) fused polypropylene fabric containing 2% (w/w) CO particles offering physical filtering performance of the mask and serving as a blockage layer; (C) inner layer made of polyester and used to mold the mask; and (A) outer layer the same as the inner layer (C). Without affecting the physical properties of the copper oxide, it acts as a potent anti-influenza biocidal agent when applied to a breathing mask (Zhong et al., 2020). Zhong et al. used laser induction to create a graphene layer over a typical surgical mask that kills viruses with electrothermal characteristics (Borkow et al., 2008). Li et al. (2019) reported a mask with two layers of non-woven fabric as biological sterilization in the intermediate layer MO Filter. The sterilization performance of the N95 mask is excellent and does not interfere with adsorption. Tang et al. sun-induced antiviral cotton fabrics made from cationic cotton fibers and anionic photosensitizers were durable and reusable and were used for face masks and protective garments. In the middle

TABLE 3 Applications of magnetron sputtering for antiviral coatings on various substrates.

MS mode	Substrate	Coating material	Application/virus type	Ref.
Radiofrequency (RF) magnetron sputtering	Soda–lime glass	Amorphous TiOx	Baculovirus	Mittireddi et al. (2021)
Direct current magnetron sputtering	PET	Cu	SARS-CoV-2	Jung et al. (2022)
Direct current magnetron sputtering	PP	Cu	SARS-CoV-2	Jung et al. (2021)
Direct current magnetron sputtering	Thermally oxidized Si (Si/SiO_2)	Cu and Ag	SARS-CoV-2	Meister et al. (2022)
RF mode for silica and DC mode for Ag	Metallic fiber-based air filters and cotton textiles	Silver nanoclusters/ silica composite	Respiratory syncytial virus (RSV), human rhinovirus (HRV), and influenza virus type A (FluVA)	Balagna et al. (2021)
DC magnetron sputtering	Cotton	Cu/Zn	Enterovirus 71 (EV71)	Zhang et al. (2022)
DC magnetron sputtering	A blend (90% polyester, 10% polyamide) and 100% cotton	Cu	Vaccinia virus (VACV), herpes simplex virus type 1 (HSV-1), and influenza A virus H1N1 (IFV)	Cieślak et al. (2022)

adsorption layer of the mask, antiviral materials were added. The hydrophobic and hydrophilic layers are the upper and lower layers, respectively. Moisture permeability, surface moisture, and resistance can all be improved using materials with high wettability, resulting in a disease-resistant and comfortable mask. For up to 60 min of sun exposure, virus substitutes (T7 pages) had a microbiome reduction rate of unevenly 5–6 logs (Li et al., 2017b).

Aside from the two main applications described above, other possibilities for infections are related to locations, for example, transportation and catering businesses. The possibilities of cross-infection can be considerably decreased if seat materials, interior textiles, seat belts, rugs, tablecloths, towels, and other textiles contain antiviral qualities (Zhang et al., 2021).

Henceforth, the spread of contagious diseases can largely be minimized by deploying antiviral/antimicrobial textiles/surfaces all over, specifically in hospital settings and high-touch public places.

7 Testing virucidal activity

International standards can be employed to ensure that disinfection products are of the highest quality and employed in the most effective strategies to prevent and control microbial contamination in healthcare settings. A book chapter that describes testing methods includes globally utilized documentation of testing techniques for virucidal activity (Woodall and Walker, 2020). A method for testing non-porous solid surfaces is recommended by the US EPA (EPA, 2016). However, the testing method for porous solid surfaces was unavailable before 2019.

Uniformity in testing procedures is important not only for comparison purposes of various reported materials but also for the commercialization of final products. Throughout the literature, scientists have used different approaches to test their developed antiviral products, and therefore, it is hard to compare the efficiency. However, ISO: 18184 is currently available for testing non-porous materials/textile products. Hence, it is anticipated that the upcoming literature will have data that could be employed to find reliable comparisons. Due to the lack of standardized protocols before the ISO 18184 standard, it is also hard to compare various surfaces reported in the literature (Walji and Aucoin, 2020).

Only a few methods have been documented in the literature aside from internationally accepted norms. An easy technique is presented, for instance, for vacuum-freezing viruses on an inanimate surface (coverslips). For the virucidal assay of disinfectants, coverslips are exposed to a medium mimicking the disinfectant (viral control) or disinfectant while upright in an Ultra-VU cuvette. The method also recommended the calculation of the means of the titers after each experiment. With this straightforward method, repetitions could be carried out with ease (Allen et al., 1988).

Another method reported the significance of large-volume plating (LVP) (Kampf et al., 2020). In this method, "sample volume" should be properly defined, whether it is a diluted or an actual sample. This report discussed materials and procedures for the *in situ* detection of living microorganisms on surfaces, specifically hard surfaces. The presence of the target microorganism is detected using the bacteriophage's specificity for a target host organism or microbe. This patented report avoids the shortcomings of sampling and off-site detection by relying instead on *in situ* detection of the target microorganism. It does not require surface sampling processes or laboratory-based detection methods (Voorhees, 2011). Recently, a method has been suggested to use bacteriophage phi 6 as a substitute for SARS CoV-2 (Serrano-Aroca, 2022). The use of bacteriophage

phi 6 allows carrying experiments even to the researcher who does not have access to BSL3 laboratory facilities.

8 Conclusion

Protection of high-touch surfaces (porous and non-porous) against the spread of viruses has become crucially important after the impact of COVID-19. Existing practices and policies require revision and quick implementation of revised policies and procedures. Disinfection must be taken as a holistic approach. In addition to commercially available disinfectants based on alcohol, quaternary ammonium compounds and aldehyde can effectively be combined in a dual strategy with solid copper alloy surfaces to reduce microbial contamination.

Copper, silver, zinc (their oxides), and TiO_2 are the most common types of metals reported to produce nanoparticles. Iron has also shown activity, but reports on iron are limited. Compared to metals, metal oxides have shown better response. Copper-based nanoparticles in small doses show faster kinetics in the deactivation of viruses. The mode of action for metallic nanoparticles is attributed to the attachment of spike proteins, production of free radicals, cell lipid layer destruction for enveloped viruses, and the hydrophobicity of nanomaterial-treated fabric. Enhanced activity of copper oxide may be due to the inherent multiple characteristics of copper, such as redox, catalytic (OH production), charge, and stability, in addition to the properties due to the nanosize of particles.

Among organic nanostructures, fullerenes, graphene, and graphene oxide and their functionalized derivatives are reported. The results of molecular docking revealed that graphene oxide sheets interact with three types of structure present in SARS-CoV-2 (i.e., closed state -6VXX or open state -6VYB (viral spike), open state -6VYB or closed state -6VXX (ACE2-bound spike complex) and 1R42 (ACE2). Graphene oxide showed good binding affinities toward all three surface structures 6VXX, 6M0J, and 6VYB.

Modifications of graphene oxide with various carbon chain lengths were synthesized and investigated. It is found that an alkyl chain greater than C9 can disturb the replication of coronavirus by disturbing its envelope. At first viral envelope is damaged when it interacts with graphene oxide, which leads to the production of ROS and virus deactivation. The lower surface energy of certain materials, along with roughness at the nanoscale, can play a great role in increasing the antiviral properties of materials; especially in the case of treated fabric or PPE, it can increase antiviral properties and superhydrophobicity.

Furthermore, among organic nanoparticles, coating of fullerene derivatives on the surface can prevent the possible attachment of SARS-CoV-2 because it produces lipid peroxidation on the lipid layer of SARS-CoV-2 and creates a hydrophobic surface that minimizes the contact between the surface and the virus due to the air entrapped between topographies.

Another important group of antiviral nanoparticles is photocatalytic materials. TiO_2 nanoparticles deposited on the surface of the glass or another inanimate surface have shown excellent virus deactivation under UV light. However, this approach would be limited to surfaces exposed to UV light and may not be effective in indoor places. Among emerging materials, g-C_3N_4 may be a promising candidate as a photocatalyst-based virucidal compound. Films of g-C_3N_4 have shown a response time of 2.4 min in killing certain microbes.

Stable and convenient methods for the deposition or incorporation of nano-antivirals on solid surfaces are necessary for the scaled production of antiviral surface coatings in the public domain. Various industrially adopted methods, for example, dip-coating, pad-dry-cure, spray coating, and magnetron sputtering methods, are tested and have shown some success. The first two methods are suitable for producing textiles, whereas the latter technique is useful for non-porous surfaces such as glass and steel. Most methods reported to incorporate nano-antivirals using dip-coating and pad-dry-cure have employed linkers (3-glycidyloxypropyl, chitosan, and monoethanolamine) for stabilizing the coating for multiple wash cycles. In a report, graphene oxide (GO) and cuprous oxide (Cu_2O) were produced and uniformly coated on polyethylene terephthalate (PET) fabric with excellent adhesion using dopamine.

Sonochemical methods are becoming increasingly popular; however, they are still not found in regular textile industry setups. In the reported literature, sonochemical methods are considered faster than other methods for depositing nanoparticles on textile surfaces. In the reported sonochemical methods, linkers are also used to attach nano-antiviral on the fabric surface. However, there is a report without the use of any linker. Cellulose's mechanochemistry is linked with the linker-free deposition of Ag/Au nanoparticles in terms of the breaking of glycosidic bonds and the production of mechano-radicals.

Copper has proven itself, whereas other virucidal materials are on the way. Copper has many faces in medical treatment and may be "*New Gold*" in solid self-sanitizing solid surfaces either as innate metal or surface coatings.

It may be concluded that the spread of contagious diseases can largely be minimized by deploying antiviral/antimicrobial textiles/surfaces and can be employed specifically in hospital settings and high-touch public places. Copper-based nanoparticles have great potential, and research should be pushed to develop eco-friendly, less expensive, and scalable approaches for the commercialization of nano-antiviral-based products.

Author contributions

FSH contributed to the drafting and literature survey of the first four sections, figure drawing, and harmonizing the whole draft. NQA contributed to the rest of the draft manuscript. NM conceived the idea and polished the draft. SQM contributed to the structure of the draft and adding tables. NA improvised the language and added a section.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The emerging significance of nanomedicine-based approaches to fighting COVID-19 variants of concern: A perspective on the nanotechnology's role in COVID-19 diagnosis and treatment

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COVID-19, one of the worst-hit pandemics, has quickly spread like fire across nations with very high mortality rates. Researchers all around the globe are making consistent efforts to address the main challenges faced due to COVID-19 infection including prompt diagnosis and therapeutics to reduce mortality. Conventional medical technology does not effectively contain the havoc caused by deadly COVID-19. This signals a crucial mandate for innovative and novel interventions in diagnostics and therapeutics to combat this ongoing pandemic and counter its successor or disease if it were ever to arise. The expeditious solutions can spring from promising areas such as nanomedicine and nanotechnology. Nanomedicine is a dominant tool that has a huge potential to alleviate the disease burden by providing nanoparticle-based vaccines and carriers. Nanotechnology encompasses multidisciplinary aspects including artificial intelligence, chemistry, biology, material science, physical science, and medicine. Nanoparticles offer many advantages compared to larger particles, including better magnetic properties and a multiplied surface-to-volume ratio. Given this, the present review focuses on promising nanomedicine-based solutions to combat COVID-19 and their utility to control a broad range of pathogens and viruses, along with understanding their role in the therapy, diagnosis, and prevention of COVID-19. Various studies, reports, and recent research and development from the nanotechnology perspective are discussed in this article.

KEYWORDS

COVID-19, nanomedicine, nanotechnology, pandemic, SARS-CoV-2

Introduction

Infectious diseases present a global threat to mankind after a disaster of cataclysmic proportions or a nuclear war. Infectious diseases are the main reason for increasing the morbidity rate, plaguing humanity, and shaping human evolution in the process (Bloom and Cadarette, 2019) and are one of the most important agents leading to premature death, especially in the developing world (Chandler, 2019). Modernization has made mankind complacent about the threat due to the increased access to medical healthcare, vaccines, and sanitization techniques. Sentinel organizations and epidemiological experts have time and again provided abundant warnings about the catastrophe related to infectious disease. Microorganisms do not need a visa to travel to different parts of the world and do not understand geopolitical boundaries. Furthermore, climate change and globalization have aggravated the concern about the spread of infections and pathogens. The newest epidemics such as Ebola, SARS, H1N1, and MERS were an alarming signal for the entire world that the viral pathogens pose a serious threat globally (Vazquez-Munoz and Lopez-Ribot, 2020). The recent pandemic that has hit the entire world is coronavirus disease, commonly known as COVID-19, caused by the SARS-CoV-2 virus (Kaushik, 2021). The virus can be transmitted directly from an infected patient *via* coughing, sneezing, and physical contact. Major symptoms of COVID-19 include fever, sore throat, sneezing, coughing, breathlessness, and tiredness (Figure 1). Moreover, patients having any secondary ailment such as blood pressure, coronary heart disease, or diabetes are potentially at high risk (Rashidzadeh et al., 2021).

Ever since the coronavirus was declared a pandemic on 11 March 2020, many new variants, such as Omicron, Delta, and many more rapidly spreading variants of concern, have posed a major challenge (Cucinotta and Vanelli, 2020). COVID-19 impacted the whole world with the economies collapsing. The first case was observed in December 2019 in Wuhan, where pneumonia-like symptoms were observed in locals. In the first week of January 2020, this new virus was found to be like coronavirus (Ahmad et al., 2020; Sodhi and Singh, 2022).

The surge in cases was exponential further suggesting that the transmission occurs *via* sneezing and coughing from asymptomatic and symptomatic patients. The coronavirus remains on the surfaces for a long period if not destroyed using disinfectants (Koo et al., 2020). As of October 2022, at least 629 million COVID-19 cases and 6.59 million related deaths had been reported globally according to the World Health Organization (https://www.who.int). A certain degree of structural homology and sequence similarity between SARS-CoV-2, SARS-CoV in 2002, and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 was identified (Sadeghi et al., 2021). SARS-CoV-2 transmissibility is higher than that of other viruses (Saylan and Denizli, 2020). The disease severity varies from mild respiratory illness to acute respiratory syndrome affecting the lungs (Chhikara et al., 2020). Infections of COVID-19 can affect the cardiovascular system (Widjaja et al., 2021), kidneys (Esmaeilzadeh et al., 2021), central nervous

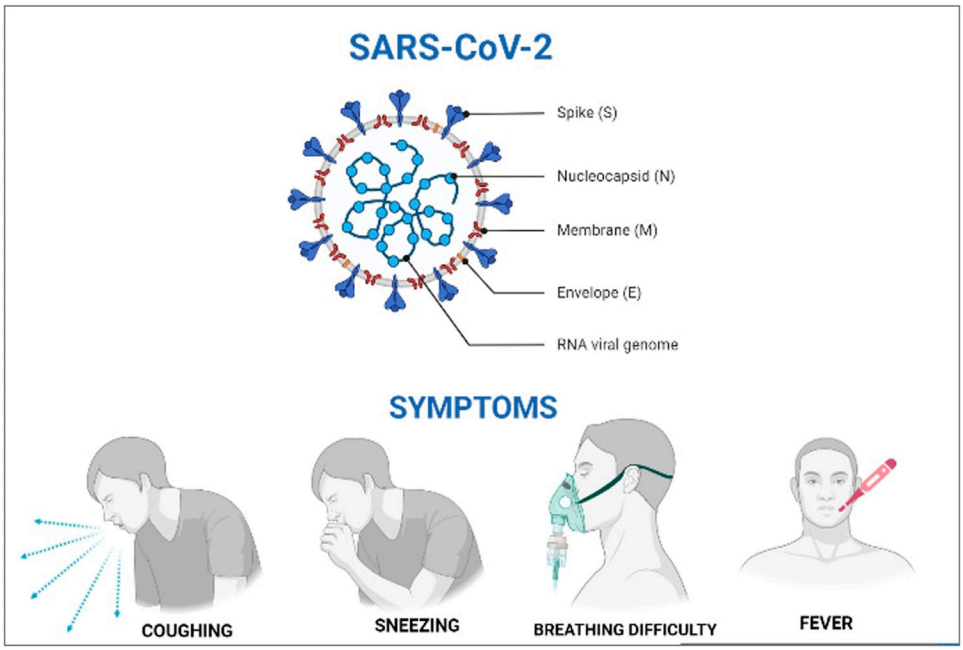

FIGURE 1
Structure of SARS-CoV-2 and the associated symptoms.

system (Esmaeilzadeh et al., 2021), gastrointestinal tract, and liver (Gavriatopoulou et al., 2020). An uncontrolled cytokine storm can lead to the failure of multiple organs, strokes, and heart failure (Rabaan et al., 2021). Many strategies for controlling the infectious disease have emerged, such as lockdowns, social distancing, quarantine, various disinfection protocols, and antiviral treatment methods (Bhaskar et al., 2020).

The conventional treatments of viral pathogenic infections can fade away due to the emergence of novel strains of the virus which emerge because of mutations in the virus (Strasfeld and Chou, 2010). Certain antiviral drugs can be used for the treatment of new strains of viruses (Jackman et al., 2016). However, novel drugs take an extended time to be approved for their safety and efficacy, so the development of new drugs is lagging (Chen et al., 2020). Multidisciplinary research is required for novel approaches for antiviral treatments along with alternative antiviral therapies, targeting the different phases in viral replication (Revuelta-Herrero et al., 2018; Mohammadi Pour et al., 2019). Nanotechnology harnessed immense attention and is explored for possible use in the treatment of viral infections (Szunerits et al., 2015; Singh et al., 2017; Lembo et al., 2018).

The unexpectedness of the SARS-CoV-2 outbreak has retarded the modalities to regulate the damage. The policies including social distancing, unprecedented strict lockdown, vaccination, and wearing face masks have been introduced to control the contagious disease (Chaudhary et al., 2021). Research is focused on modeling innovative point-of-care practices for timely diagnosis and therapeutics to control pandemic-initiated mortalities and morbidities. These strategies are employed, but their success varies spatially and is influenced by environmental factors. Various environmental factors, such as relative humidity, ammonia concentration, and particulate matter, are responsible for the spread of COVID-19 and associated mortality (Bollyky et al., 2022). In the study of Chaudhary et al. (2022a), correlation analysis was used to study the role of environmental factors in the progression and severity of COVID-19. A statistical analysis indicates the correlation between COVID-19-associated mortalities and various environmental factors. The regression model throughout the strategic lockdown has suggested the prominent triumph of unparalleled constraints in Delhi in the form of restrictions on movement. Particulate matter is anticipated to be an important risk promoting the severity and outbreak of COVID-19. The hotspot mapping of airborne ammonia, particulate matter, and relative humidity was also identified using the regression model and mapping the ambient concentration. Areas in the capital undergoing rapid construction, industrial activities, and vehicular emissions are the major hotspots.

Modernization and urbanization can increase the risk of many unseen pandemics in the future. COVID-19 has shaken the world, but still, efficient antiviral treatment options are lacking. COVID-19 presents three major challenges: timely diagnosis, prevention, and treatment (Chaudhary et al., 2021). The major concern worldwide is the determination of an outbreak at an initial stage, which has led to the development of strenuous efforts in the development of point-of-care diagnosis, especially the advanced rapid test, tests based on artificial intelligence, and computed tomography (CT) scans. Forthcoming measures of detection, cure, and prevention have been extensively researched, and advancements have been made using nanotechnology (Chaudhary et al., 2022b). Diverse formulations based on the concept of nanotechnology are considered promising for vaccine development (Cascella et al., 2022). Delivery of nanomaterial-based formulations commends the active agent concentration, silencing RNA, vaccines, antiviral species, and antibodies at the sites of infection generating a basic potentiation of the immune system (Ruiz-Hitzky et al., 2020). Biohybrid nanomaterials are based on polysaccharides and fibrous clay assembly and show a promising environment for viral particles, aiding in their binding to the biohybrid material and helping in conserving their bioactivity. Nanoparticle-based formulations are used in vaccination and for intranasal administration for coronaviruses and influenzas (Al-Halifa et al., 2019).

Nanotechnology has a lot of potentials, and it can be used for the development of medicines and drugs at the nanoscale and can be utilized in the development of novel approaches to deal with viral infections. This can be proved to be a cutting-edge technology that can be used for the successful treatment of COVID-19, but it can also be used to improve the already existing diagnostic and therapeutic strategies (Chintagunta et al., 2021). Nanotechnology is the application of devices and materials in which at least a single dimension is lower than 100 nm. The nanotechnology's application in medicine is called nanomedicine, which comprises the nanomaterials used in the control, prevention, diagnosis, and treatment of diseases (Yayehrad et al., 2021). Nanoparticle research has gained much attention in the last decade due to the advantages of nanoparticles such as small size, multifunctionality, better solubility, adaptability, production of better and safer drugs, nanomedicines, and early diagnosis of diseases (Bhavana et al., 2020; Pandian et al., 2021).

Nanotechnology can help combat COVID-19 by avoiding viral contamination by designing nano-based vaccination for boosting both humoral and cell-mediated immunity, designing personal protective equipment (PPE) kits and disinfectants to increase the safety of front-line workers, designing sensitive and specific nano-based sensors for quick immunological response, and developing novel drugs, having enhanced antiviral activities, sustained release, tissue specificity, and reduced toxicity (Balkrishna et al., 2021).

Nano-formulations can be used to enhance the target delivery and therapeutic efficacy of the antivirals (Singh et al., 2017; Lembo et al., 2018). There is a lack of therapeutic choices for the treatment of viral infections, so plant metabolites offer a

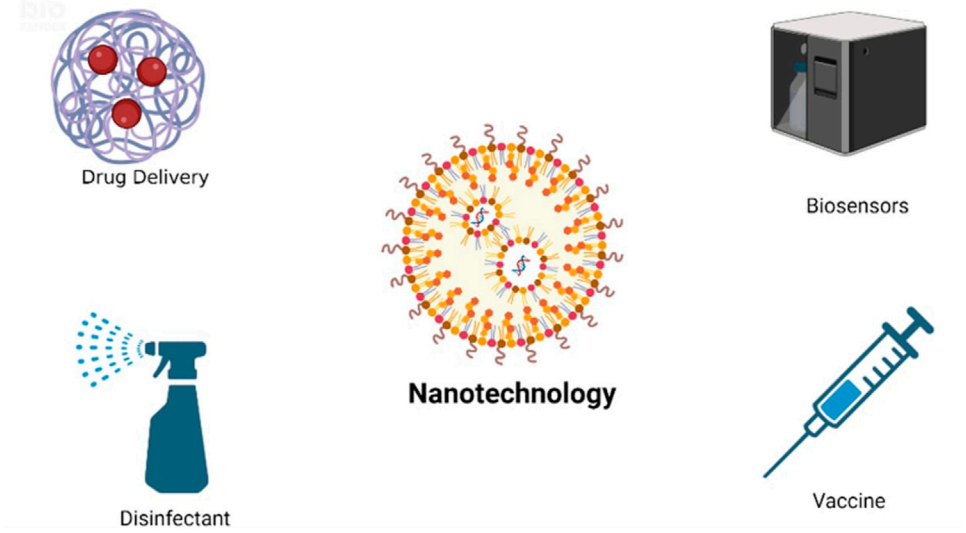

FIGURE 2
Applications of nanomedicine to fight COVID-19.

lucrative option for their treatment. However, plant metabolites have poor solubility and availability, so they are often combined with nano-based carriers for better therapeutic effects (Watkins et al., 2015; Gera et al., 2017; Praditya et al., 2019). Not only in therapeutics but also in diagnostics, many nano-based sensors are used that have high sensitivity and specificity (Mokhtarzadeh et al., 2017). Next-generation vaccines are also available which are based on nanomaterials, offering improved antigen specificity, control release, and target delivery (Vijayan et al., 2019). Delineation of mechanisms involved by which the virus infects the host cell is also carried out (Campos et al., 2020). Figure 2 shows the applications of nanomedicine to fight COVID-19.

Many studies have reported nanotechnology used in the treatment of chronic illnesses such as cancer, but very few studies report their use in addressing the challenges imposed by COVID-19. So, given this, the present review critically discusses various nanotechnology-based diagnostic and therapeutic strategies for managing the SARS-CoV-2 variants of concern.

Role of nanotechnology in COVID-19 diagnostics

Nanostructured systems aid in the advancement of COVID-19 detection, increasing their sensitivity and specificity for signal amplification in a reverse transcription–polymerase chain reaction (RT-PCR) and prophylaxis for vaccines as adjuvants (Krishnan et al., 2021). Nanoparticles play a therapeutic and vital role, especially during different stages of COVID-19 pathogenesis because of the inhibition potential for the preliminary attachment and membrane fusion when the virus enters and in the protein fusion of the infected cell (Vahedifard and Chakravarthy, 2021). Nano-encapsulated antiviral drugs are more effective in the activation of the intracellular mechanism which leads to irretrievable harm to viruses and inhibits the cellular machinery of the viruses by impairing their replication, transcription, and translation. Novel nano-technological methods can help detect COVID-19 infection. A few problems are associated with the already existing protocols; for example, the RT-PCR used for testing the asymptomatic COVID-19 patients was not available in many non-urban centers. The WHO stated the urgency for novel diagnostic kits for detecting SARS-CoV-2 (Kamat et al., 2021; Fernandes et al., 2022).

COVID-19 detection using loop-mediated isothermal amplification

Loop-mediated isothermal amplification is a rapid method that has high specificity and sensitivity (Kim et al., 2022). Xu J. et al. (2020) showed that magnetic nanoparticles can be used in RT-PCR diagnosis and for extracting SARS-CoV-2 RNA. Magnetic nanoparticles are used in this technique which are functionalized with poly amino ester, and the extracted RNA was absorbed on magnetic nanoparticles containing carboxyl groups. The interaction of the RNA magnetic nanoparticle and carboxyl groups is used for the analysis (Xu L. et al., 2020). They are directly used without the elution of viral RNA from the magnetic beads. It is a time-saving mechanism and protects from any

contagious risks. The magnetic nanoparticles were used by the same group in a different study, where the virus was isolated instead of eluting viral RNA. The enriched viral particles are bound tightly to nanoparticles because of target receptors. The magnetic nature of the nanoparticles is attained by the encapsulation of paramagnetic iron oxide. They help in the detachment of the virus from nanoparticles with the aid of an external magnet and detect the virus by immunochromatographic strip tests, cell-based tittering assays, and quantitative RT-PCR (Mujawar et al., 2020; Shan et al., 2020).

Fifth-generation biosensors for COVID-19 detection

Current health crises because of infectious diseases including COVID-19 and monkeypox have raised the model of multifunctional and portable biosensors embedded in the solo chip. Conventional diagnostics methods are costly, complex, and time-consuming; therefore, biosensors are used as a lucrative alternative as they possess applications in the pharmaceutical and healthcare industries (Verma and Bhardwaj 2015). Biosensors are installed for the recognition of multiple diseases, prevention, health observation, and rehabilitation of patients (Dwivedi et al., 2021). The fifth-generation biosensors can be used for studying advanced nanomaterials, and they can be integrated with intelligent and rapid data processing strategies and packed in portable modules for various healthcare applications (Wu et al., 2022; Zhang et al., 2021). Biosensors aid to integrate the pioneering functional materials having manageable physicochemical attributes and ideal machine processability. The 2D metal nitrides and carbides show promising performances due to their adjustable physicochemical properties and rich surface functionalities. The biosensor hybridization along with diversified nanomaterials provides challenges for the commercialization of stability due to oxidation. Biosensors have been interfaced with modern-age technologies, including 5G communication and artificial intelligence (AI), for heading toward the hospital-on-chip (HOC) modules. Graphene and its derivatives, metal nitrides and carbides (MXenes), and borophene have emerged as excellent biosensing platforms with a high specific surface area with enhanced detection and monitoring efficacies (Zhang et al., 2021; Chaudhary et al., 2022a; Chaudhary et al., 2022c). MXenes demonstrate enormous potential for monitoring and detecting diverse biomolecules and encompassing electrochemical, optical, and plasmonic modules (Sheth et al., 2022). The infectious and fatal diseases have certainly burdened the already existing healthcare services globally and resulted in mortalities, so the primary concern in the post-COVID-19 era is the early diagnosis of contagious diseases to strengthen their treatment and curtail the spread (Chaudhary et al., 2022b; Noh et al., 2022). The consequences of infectious diseases can be managed *via* early diagnosis of respective biomarkers, so as to enhance their therapeutic efficiency (Cherusseri et al., 2022; Markandan et al., 2022). Timely detection *via* point-of-care (POC) diagnostics is highly advantageous, as it is highly efficacious and simple. Nanomaterials can also be utilized to enhance the functioning of the POC devices for improved efficiency. Table 1 and Figure 3 show the use of nanotechnology in the prompt diagnosis of COVID-19.

Nanomedicine's role in COVID-19 treatment

Nanomedicine influences every field of medicine and is a dynamic tool for the development of novel therapeutics, medical imaging, nanotherapeutics, vaccines, and biomaterials for the regenerative medication (Varahachalam et al., 2021). Nanomedicine is an amalgamation of nanotechnology and medicine and uses nanoparticles in therapeutic or diagnostic applications (El-Sayed and Kamal, 2020). Soft nanomaterials are attained from lipids, proteins, polymers, and surfactants and are often applied in nanomedicine for drug delivery. There are also prerequisites for the use of nanomaterials (Devadasu et al., 2013). Several drug-based nanoparticles are under clinical trials for diseases such as neurodegenerative diseases, cancer, cardiovascular, infectious, and inflammatory illnesses (Yetisgin et al., 2020). Nanoparticles can aid in active or passive drug targeting along with controlled drug release, which affects the safety and efficacy of the treatment. Metal nanoparticles can be used in nanomedicine, due to their antibacterial, antiviral antifungal, and antiparasitic activities (Singh et al., 2019). Pathogenic bacteria develop resistance to already used antimicrobials, which led to the development of nanotechnology-based antimicrobial therapy, such as metal-based antimicrobials, which are effective in the treatment of superbugs. Similarly, the incidence of a new strain of viruses and heterogeneity has likewise necessitated advanced and innovative therapies. Since nanotechnology offers specific targeting, it can be used for antiviral therapy (Teixeira et al., 2018).

Nanoparticles can be used to combat SARS-CoV-2 by targeting mechanisms that affect the viral entry inside the host until they get activated. By blocking the surface proteins on the virus, the virus gets inactivated, so nanoparticles are designed specifically for virus-expressed proteins and could diminish viral internalization (Goscianska et al., 2022). Metal nanoparticles can block the virus's attachment to the host cell, thereby inhibiting the internalization of the virus and impairing the replication of the virus during viral entry. Silver (Ag), titanium (Ti), zinc (Zn), and gold (Au) have shown results against the influenza virus, herpes simplex virus, transmissible gastroenteritis virus, Zika virus, HIV, and monkeypox virus (Mainardes Diedrich, 2020).

TABLE 1 Use of nanotechnology in the prompt diagnosis of COVID-19.

S. No.	Technique	Application	Reference
1	Point-of-care testing	• Used to diagnose infected patients without transporting the samples to the laboratory	Konwar and Borse (2020); Xu J et al. (2020); Asif et al. (2020)
		• Colorimetric biosensor centered around paper-based DNA is used for detecting the virus very rapidly in the sample	
		• The paper-based sensor uses a cationic pyrrolidinyl peptide nucleic acid (PNA) having better hybridization than RNA and DNA probes. The presence of lysine in the probe imparts a positive charge and interacts with the silver nanoparticles along with negatively charged DNA	
		• PNA particles can bind to the silver nanoparticles causing the aggregation of the nanoparticles if viral RNA/DNA is not present, whereas when viral RNA/DNA is present, a complex is formed with the COV virus without any nanoparticle aggregation	
2	Chiral biosensors	• Chiral zirconium quantum dots are utilized for COVID-19 detection. The formulation contains zirconium quantum dots, magnetic nanoparticles, and antibodies specific to the coronavirus	Ahmed et al. (2017)
		• The magnetic nanoparticles and zirconium dots bind to the virus strongly, and magneto-plasmonic fluorescence is displayed if the virus is present	
		• The external magnets are used to separate the magneto-plasmonic-fluorescent nanohybrids to measure the fluorescence intensity to detect the presence or absence of the virus	
3	Electrochemical biosensors	• A slight modification of the electrochemical sensing process is performed using the gold nanoparticles which aid to retain the functional moiety of the biomolecule	Martínez-Paredes et al. (2009); Ishikawa et al. (2009)
		• Gold nanoparticles play a role in the interface as it acts as an electrocatalytic material	
		• Carbon electrodes are utilized to coat the gold nanoparticles and as an immunosensor in one of the studies, where immobilization of spike S1 protein of COVID-19 is performed using the gold nanoparticles and the immobilized protein can interact with the SARS-CoV-2 in the sample and can bind to the antibody in the sample	
		• In the absence of SARS-CoV-2, the antibody in the sample can bind to the spike protein and a reduction in the peak current can be observed, but in the presence of the virus, the antibody in the sample will bind to the immobilized antigen	

Organic nanoparticles are used to improve the bioavailability of the antivirals such as dapivirine, zidovudine, efavirenz, and acyclovir. The organic nanoparticles are also important to promote effective drug delivery and help in targeted antiviral delivery (Delshadi et al., 2021). Nanoparticles overcome antiviral limitations to specific targeting which results in cytotoxicity of the host cell. Many clinical trials have been conducted for COVID-19, and antimicrobials like ribavirin, lopinavir, remdesivir, chloroquine, and ritonavir were tested and showed promising results against SARS-CoV-2 (Oroojalian et al., 2020). Nanoencapsulation of these drugs may aid in the development of safer treatments for viral diseases such as COVID-19. The nanoparticles in the case of viral diseases are underused and not fully explored, which came to light during the pandemic (Pandey et al., 2020).

Nanomedicine can deliver a general platform that can be improved simply to ensemble the application in necessity. This can be displayed during this brief span since the pandemic started. For example, with the change in the therapeutic particle encapsulated inside synthetic or natural nanoparticles, a different vaccine for the treatment of COVID-19 can be developed. Specific regulations are lacking for the approval of

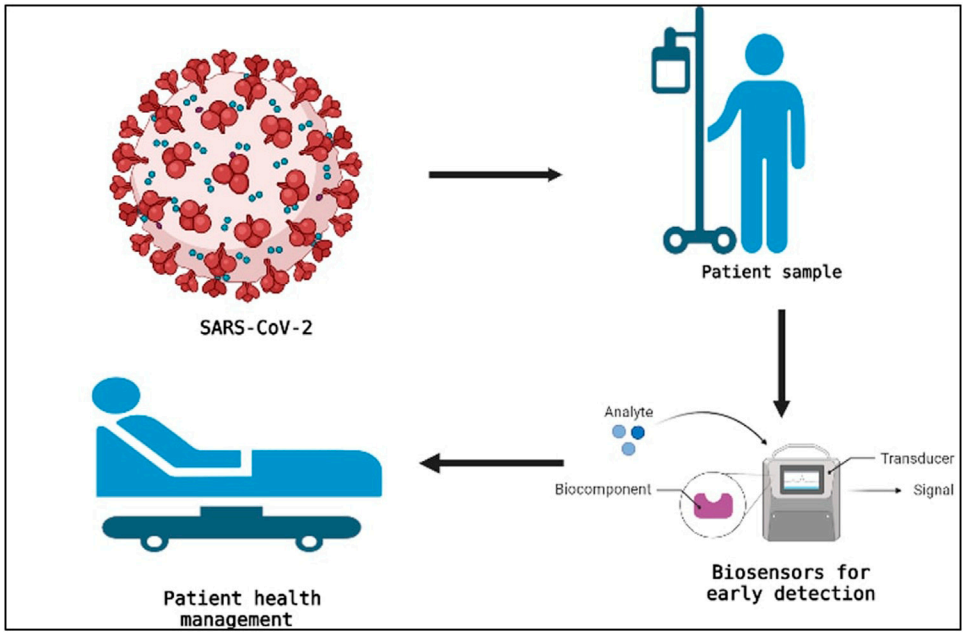

FIGURE 3
Rapid diagnosis of COVID-19 using biosensors.

nanomedicine, and novel nanomedicine must undergo full clinical trials (Germain et al., 2020). During the hard times amidst the coronavirus, nanomedicine has outshined other fields, and the knowledge has provided science with innovative therapeutic modalities for the pandemic. The clinical development of nano-products is necessary to solve the clinical needs which are beyond oncology. It is vital to report the lacunae facing the development of nanomedicine such as aspects, especially related to the nanotoxicology of new nanomaterial classes along with the global explanation on monitoring approval for both the biological activity and physicochemical characterization, especially for multifunctional products (Hashemi et al., 2021).

Nanomedicine with a size of up to 200 nm is a beneficial cargo fabricated using a suitable drug nanocarrier as a beneficial agent (Moghimi et al., 2005; Karimi et al., 2016). Magnetic nanoparticles can be used to control and manage the virus pathogen even in the brain as nanomedicine can cross any barriers *via* the following measures: 1) nanomedicine functionalization with specific receptors for the brain, 2) using ultrasound as external stimulation, and 3) a non-invasive method which can use a magnetic drug delivery system (Mitchell et al., 2021; Mohammadi et al., 2022). Magnetic nanomedicine is designed to deliver the drugs at a specific site to control release by applying peripheral stimulation like a magnetic field, and the drug release sequence can be strategized according to the prerequisite of the disease condition (Ashwini et al., 2022; Naghdi et al., 2022).

The nanomedicine performance depends mainly on the choice of a multifunctional stimuli-response drug nanocarrier, opto-magnetic, magneto-plasmonic nanosystem, and magneto-liposome (Kaushik, 2021). Nanomedicine can be vital for the recognition of disease progression and drug distribution. Manipulative magnetic nanomedicine (MMN) is a possible upcoming therapy wherein control over delivery and routine is mandatory (Kaushik, 2021). MMN can aid in the identification and elimination of the SARS-CoV-2 virus for the management of viral symptoms and infection. In addition to the elasticity in using the therapeutics of the choice, these unscrupulous nanomedicines can be recognized as a long-interim treatment for the SARS-CoV-2 infection, where immune supportive agents can stay for a long period in the body without fabricating any ill effects. MMN proves to be a compulsory remedy, and emphasis on its expansion must be made by the forthcoming study with the resulting objectives: 1) exploration of stimuli-responsive MMN for precise delivery and release; 2) confirmation of the site of drug delivery and release by using image-guided therapy; 3) delivery of drug in the gut and blood–brain barriers using the magnetic guided approach; and 4) the customization of MMN according to the medical history and disease profile of the patient, for example, selection of CRISPR-Cas and anti-SARS-CoV-2 virus based on the genomic profiling of the patient (Kaushik, 2021). The customization of the MMN for extended therapeutics allows the release of the drug for a longer time to manage post-COVID-19 effects (Jayant et al.,

2018; Kaushik et al., 2018; Kaushik et al., 2019; Tomitaka et al., 2019; Kaushik, 2021).

SARS-CoV-2 infection is a manifestation of many symptoms. COVID-19 infection requires extensive treatment, and even after discharge from the hospital, the patient may have the complicated condition for a longer time (Dao et al., 2021). Conventional antiviral drugs cannot be used for the treatment of adverse symptoms. Micro-needle-based vaccine delivery is used for the management of COVID-19 infection. Initial trials are promising, but a lot more must be carried out for animal-based trials, followed by FDA approval. MNM can be promoted against COVID-19 effectively, and research is required in this field to traverse the path from *in vitro* to *in vivo* and finally to clinical and human trials, and FDA approval is required for public use. For the development of anti-COVID-19 infection therapy, critical safety-related risk assessments should be assessed. Artificial intelligence introduction can be a good option, because of the use of bioinformatics to perform big data analysis and avoid hit-and-trial approaches (Gage et al., 2021; Kaushik, 2021).

Artificial intelligence-based face masks to combat COVID-19

Since the old civilizations, face masks are a vital component of human lives. Face masks are used as an economically viable tool and create a sense of communal unity and combat health hazards (Chaudhary et al., 2022d). The use of face masks is still not normalized because of manufacturing limitations, solid waste production, and unawareness. The limitations have been overcome by the choice of material utilized for manufacturing (Ganesapillai et al., 2022). Masks are designed from textiles such as biomaterials and nanomaterials in the present-day market. Face mask respirators (FMRs) are engineered using nanomaterials and are advantageous as they have a high specific surface area, unique physicochemical properties, multiple usages, good breathability, and pathogen-detecting and -scavenging capabilities (Forouzandeh et al., 2021). FMRs are vital as they break the chain of viral infection, thereby protecting the community spread (Chaudhary et al., 2022a). The SARS-CoV-2 viral infection is correlated with fungal and microbial infection. Mucormycosis spread in various countries is a consequence of COVID-19. The variants of concern like Omicron have a higher spread rate and have challenged the diagnosis, prevention, and therapeutics for COVID-19 (Bhatt et al., 2021). More novelties, especially in designing the preventive FMRs are crucial with intelligent and smart features. The inclusion of the contemporary technologies of nanotechnology, Internet-of-Things (IoT), artificial intelligence (AI), and machine learning can be useful in architecting the FMRs (Umapathi et al., 2021). The IoTs can be used and integrated with nanomaterials for the fabrication of next-generation FMRs having AI techniques. The sensors can also be incorporated into the FMRs to observe human behavioral and physiological signals. Even in the remotest part of the world, nanomaterial-based biosensors can be integrated into FMRs using minute radio antennas used for the diagnosis of COVID-19 (MacIntyre and Chughtai et al., 2020). The radio antenna integrated into the FMRs can aid in the recording of COVID-19 cases. The integration of AI makes the technology available to any user and helps in the management and monitoring of COVID-19. With the help of AI, smarter FMRs can be designed and can control the massive spread of COVID-19 (Manickam et al., 2022). The Global Positioning System (GPS) should be incorporated to assess the patient's routine and set up early warnings and advice (Chaudhary et al., 2022b). A smart filtering facepiece (FFP2) mask with an opto-chemical sensor integrated and driven *via* a smartphone is used for monitoring CO_2 inside the mask. This mask is fabricated specifically to tackle the health effects that arise due to prolonged use of the face mask. Chen et al. (2022) reported the use of an FMR based on electrospun polyetherimide (PEI) electret non-woven. It is a bi-functional mask that removes particulate matter and is also used for the generation of electricity. It is capable of self-sterilization and has features such as biodegradability, transparency, breathability, hydrophobicity, and biocompatibility (Chen et al., 2022).

Application of 3D printing to combat COVID-19

The demands for medical technology have exceptionally increased since the arrival of the global pandemic. Innovative 3D printing, which was initially started with no practical utilization, has now been accepted in various industries, for example, engineering and healthcare (Larrañeta et al., 2020). Three-dimensional printing is used to produce face shields, components of the ventilator, and masks which were of utmost importance during the pandemic scenario. In this technique, products are fabricated utilizing digital CAD files using layer-by-layer techniques (Munaz et al., 2016; Irfan Ul Haq et al., 2020). It is highly favored in the medical field because of its customization. This technique is beneficial as it helps in adapting to variations as per the circumstances of the patient (Marro et al., 2016). The software helps in conceptualizing medical equipment, which is later 3D-printed with less cost and time. The rapid prototyping of this technique permits a rapid mobilization of the equipment (Marro et al., 2016).

A 3D antibacterial swab made up of a bio-cellulose lattice structure is used for the detection of COVID-19 as the use of a nasopharyngeal swab poses a serious problem. Nasopharyngeal swabs which are 3D-printed deliver a lucrative and fast substitute as compared to conventional nasopharyngeal swabs (Haleem et al., 2021). Three-dimensional printing is vital for hospitals as it

TABLE 2 Nanomedicine formulations for the COVID-19 therapeutics.

S. No.	Formulation	Application	Reference
1	Nano-sponges	Nano-sponges' formulation is one significant development in the field of nanomedicine. The nano-sponges are made up of nanoparticle cores, encircled by cellular membranes. A three-dimensional network offers many advantages such as superior drug absorption and complexation and slow degradation. Molecular built-up and assembly of nano-sponges allows the drug co-encapsulation for effective and direct treatment. For example, the pore-forming toxins (PFTs) can disturb the host cell membranes and increase the permeability. It is important in bacterial infections and is a part of the virulence control mechanism	Swaminathan et al. (2016); Tannous et al. (2020)
2	Nanoparticle-based drug therapy	In nanomedicine, nanoparticle-based drug therapy is an advanced technology. It improves the therapeutic indices by, for example, increasing the immunogenicity, bioavailability, solubility, diffusivity, and controlled and targeted release of the drug *in vivo*. Copper nanoparticles can enhance the efficacy of remdesivir and arbidol which can treat COVID-19	Patra et al. (2018)
3	Nanoparticle-based gene therapy	Gene therapy deals with the replacement or deletion of "defective" genes in the unhealthy cell. Recombinant nucleic acids containing therapeutic genes result in blocking the translation of mRNA, resulting in reduced disease progression. Nucleic acids such as microRNA (miRNA) and small interfering RNA (siRNA) are used for the purpose. Clustered regularly interspaced short palindromic repeats (CRISPR) technology has reformed treatment for COVID-19. Use of Cas9, a nuclease derived from bacteria is used to fix precise point mutations on a target gene	Wei et al. (2020)
		The major challenge is in the innocuous and precise delivery of CRISPR-Cas9 to the target site, and nanomedicine is a possible solution. Copper nanoparticles are a good option for the CRISPR-Cas9-mediated gene therapy delivery to target lung tissues damaged due to SARS-CoV-2	

is remarkably positioned to elucidate the instant needs and acts quickly on them by operating together between industry and hospital and helping in saving valuable time to address the shortage needs in hospitals (Javaid & Haleem, 2021).

Three-dimensional-printed face masks are vital. One example is the Copper3D NanoHack mask which creates the borders of the community-created tactics and the necessity for the improvement of design which is based on the availability of the technical base and local testing. A polylactic acid (PLA) filament is used for 3D printing, and it is hand-assembled into the final 3D configuration when heated at 55–60°C through a hair dryer or hot air (Belhouideg, 2020). The mask consists of a simple port for air intake with the insertion of two reusable filters, and the filter can be kept in place by a screw-in cover (Tino et al., 2020). PPE is also used in 3D printing. The pandemic significantly increased the use of PPE, especially its use in healthcare has significantly increased. There is a surge in the propagation of the 3D printing method mainly due to an increase in the ability to produce PPE *via* organized means.

Three-dimensional printing offers many advantages, but there are also a few limitations to 3D printing (Clifton et al., 2020). The major problem is that the mechanical hindrance of particles can only be simulated to a degree, but the multiplication of the electrostatic properties of the filter with 3D materials is a major challenge. Thermoplastic filaments which are essential for fused deposition modeling (FDM) printing vary extremely in the composition of materials, porosity, and environmental stability (Katkar et al., 2018). FDM filaments grasp ambient moisture, which could stance an absurdly amplified risk for virus transmission during reuse (Jurischka et al., 2020). Table 2 shows the nanomedicine formulations for the COVID-19 therapeutics.

Challenges to the use of nanotechnology in COVID-19 treatment

Respiratory infections are one of the most common reasons for deaths worldwide. Although there are vaccines and drugs available now to prevent or treat COVID-19, many existing drugs are repurposed drugs that are effective against very few pathogens (Saha et al., 2020). Along with the conventional approaches, researchers are trying to develop suitable nanomedicines. This review highlights the role of nanomaterials in diagnosis, prevention, and vaccine production, and their role in the treatment of patients relapsing after finishing a conventional antiviral treatment (Yetisgin et al., 2020). Nanoparticle development is a lucrative option due to its surface charge, shape, size, large surface-to-volume ratio, and biological and functional properties. The conventional therapies do not appear to be well-equipped in handling the pandemic. Therefore, we must prioritize diversifying our research to seek a permanent solution that can fight future pandemics. Nanotechnology can help develop measures to reduce SARS-CoV-2 infectivity and help in prompt diagnosis of the contagious disease (Harish et al., 2022). Sanitizers based on nanotechnology display a broad and

potent antimicrobial and antiviral activity; therefore, they can be instrumental in improving safety and uplifting healthcare facilities, especially in developing countries (Sportelli et al., 2020). Nanotechnology plays a crucial role in the design of detection kits, vaccines, and therapeutics to fight the COVID-19 variants of concern. The therapeutic use of antiviral drugs based on nanotechnology is an effective remedial option; as more research options are accessible, it can further be expanded (Varahachalam et al., 2021). The antimicrobial potency of drugs can be enhanced using nanomaterials for the treatment of secondary infections. Nanotechnology along with *in silico* drug designing, artificial intelligence, and synthetic biology is still in a nascent stage, especially with regard to their applications. Most of the translational research on nanotechnology and other promising domains is utilized for diseases such as cancer (Kamat et al., 2021). Nanotechnology offers many limitations in managing COVID-19, as it fails to eradicate viral particles within the body. The lack of research to study the *in vivo* behavior of these nanoparticles inside the body, the immunological response to these nanomaterials and their systemic clearance, and side effects is not well understood (Rai et al., 2021). With the severity of the ongoing pandemic and the sudden damage it has caused, many nations are unwilling to risk their resources on unconventional technologies. RNA viruses are prone to mutations and recombination which makes them more dangerous to humans and can be considered a global threat (Hu et al., 2021). Nanomaterials, especially due to their nano-size can cause respiratory ailments and lung problems, and this aspect should be kept in mind while designing the nanoparticle. Oxidative stress, fibrosis, immunotoxicity, inflammation, potential cell toxicity, and genotoxicity of the nanoparticles are important issues that must be solved before reaching patients (Di Giampaolo et al., 2021).

Conclusion

Academia and research industries all over the world are working to alleviate the health crisis caused by COVID-19. Nanoscience and nanotechnology tools offer a useful approach to the present global priority. Basic research, which starts from the computational simulation to study the interaction of the virus with nanomaterials, is vital to obtain data on the viral particle's nanostructure and their mechanisms of infection. Safe and judicious use of nanomaterials is vital, as almost all the studies conducted so far have evaluated the treatment using only the *in vitro* approaches. Nanomaterials' behavior and fate can change upon reaching blood circulation. Thus, we should rely on *in vivo* models for long-term exposure to comprehend the behavior of the nanoparticles in the body. There is a need to accelerate the deployment of suitable approaches which can be used to prevent, diagnose, and treat infectious diseases. Advances in the treatment and diagnostics of COVID-19 are continuing quickly, but novel viruses such as coronavirus and their mutants are more aggressive than the typical flu. Antiviral drug cocktails helped patients with moderate COVID-19 symptoms and stopped the multiplication of the virus. The use of nanocarriers can help in the transportation of these drugs, and their administration through the nasal route could save many lives. Nanotechnology advancement provides a lucrative solution for the rapid detection of the infection and helps to develop antigen and antibody testing kits that are useful, especially for asymptomatic patients. Lipid nanoparticles, viral-based vaccines, hard nanomaterials, which are used for viral detection, and the fabrication of PPE are at the lead in the pandemic. These technologies stemming from nanomedicine are very promising and lay the platform to deal with unforeseen pandemics in the future. It is a big push for the application of nanomedical technologies and eventually becomes an incentive for industry stakeholders, funding, and regulatory bodies to invest further in this budding field. Continuous research is required to improve the characteristics of these diagnostic, preventive, and therapeutic modalities *via* the use of nanotechnology which is the primary focus of the researchers. Researchers identifying the nanomaterials for medical sciences must warrant that nanomaterials can deal with any future pandemics.

Author contributions

CKS: writing and editing. KKS: conceptualization, writing, editing, and supervision.

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Conflict of interest

The reviewer VC declared a shared parent affiliation with the authors to the handling editor at the time of review.

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Nanotechnology and COVID-19: Prevention, diagnosis, vaccine, and treatment strategies

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In December 2019, Coronavirus pandemic (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viruses, which affected the whole world, is emerged. The details on the epidemiology, infection source, transmission mode, and prognosis of SARS-CoV-2 gave in this review. Universal infection control standards such as hand hygiene, environmental cleanliness, use of personal protective equipment, and quarantine used to prevent the spread of COVID-19 without vaccine. However, many vaccine candidate studies carried out globally with using traditional and technological approaches. Innovations in technology allow the development of nanotechnological tools and the formation of systems that will inactivate SARS-CoV-2 in patients. It expected to include technologies that combine different disciplines, especially robotic applications, antimicrobial nanotechnology, and tissue engineering for the future treatment of COVID-19. This review-based work discusses the relationship of COVID-19 and nanotechnology based working principles.

KEYWORDS

COVID-19, pandemic, nanomedicine, nanotechnology, SARS CoV-2 (COVID-19)

1 Introduction

Nanotechnology is used to achieve precision manufacturing at the nanometer scale (10^{-9} m). In its most comprehensive expression, it is the most current technology that enables the production of materials to be used in chemical, physical, and biological systems in submicron sizes and the integration of the obtained nanomaterials into larger systems (Nasrollahzadeh et al., 2019). The developed nanomaterials (Figure 1) are classified into four classes according to their dimensionality. First and second classes are miniature materials (nanospheres and clusters) and one-dimensional materials (nanotubes, wires, and rods), respectively. Other classes are called as two dimensional materials (thin films, plates, and layered structures), and three-dimensional materials (bulk nanomaterials, hydrogels, and polycrystals) (Poh et al., 2018). These materials have more functionality due to their small size, and contribution for developing the physical, chemical, electrical, mechanical, and optical properties of the systems in which they are used (Lan, 2022). Also, they bring many innovations from daily life to the industrial area (Nasrollahzadeh et al., 2019). The application of nanotechnology (Figure 2) has branched out into a large number of various areas of science as environmental applications (i.e., energy conversion/storage/transmission, and water treatment), agricultural industry applications, cosmetics, and

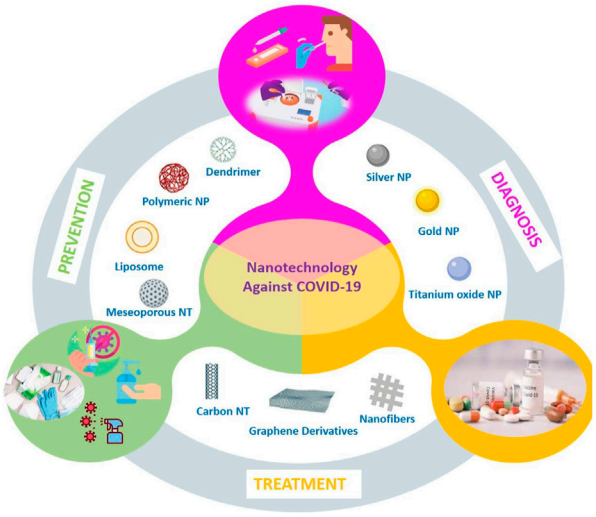

FIGURE 1
Nanomaterials are used in diagnosis, prevention, and treatment of COVID-19.

FIGURE 2
Nanotechnology is used in different scientific applications, including COVID-19.

nanomedicine (Kargozar and Mozafari, 2018). Furthermore, according to latest studies, the nanomaterials play an important role in the imaging, diagnosis, and treatment processes of diseases caused by viral infections, including SARS-CoV-2. Enzyme-based tests (Mahmoudinobar et al., 2021) and infection diagnostic kits (Singh et al., 2021) used for diagnosis of COVID-19; gloves, masks (Mallakpour et al., 2022), disinfectants (Talebian et al., 2020), and vaccines (Kashte et al., 2021) used to prevent spread of COVID-19; and drug delivery systems (Chowdhury et al., 2021) for the treatment of COVID-19. Considering the effect of the virus and the problems it causes, emergency solutions based on nanotechnology should be developed because the virus threatens the lives of hundreds of people every day (Campos et al., 2020; Ruiz-Hitzky et al., 2020).

1.1 Coronaviruses and SARS-CoV-2

The world is currently dealing with COVID-19, a serious and acute respiratory problem that started in December 2019 and is still an ongoing issue (Tavares et al., 2022). Since the disease began to spread, it has caused close to 600 million confirmed cases of COVID-19 and approximately 6.5 million deaths up to 26 August 2022 (WHO, 2022a). The disease was global pandemic in March 2020 by the World Health Organization (WHO). SARS-CoV-2 first emerged in Wuhan, China that belongs to the *Coronavirinae* subfamily (family: *Coronaviridae*) (Campos et al., 2020; Stewart et al., 2020). SARS-CoV-2 is a spherically enveloped RNA virus that leads to higher pathogenicity, contagiousness, and mortality rates than SARS-CoV, and the Middle East respiratory syndrome coronavirus (MERS-CoV) (Rossi et al., 2020). SARS-CoV-2 has structurally four main proteins as Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) proteins (Figure 3). The S protein is the glycosylated main surface protein which covers the surface and is crucial for the attachment of viruses to the host cell and entering it (Xia, 2021). The E protein is the small structural protein and is involved in viral assembly (Schoeman and Fielding, 2019; Rahman et al., 2021). The M protein is the most abundant structural protein and especially involved in the formation of the viral envelope (Schoeman and Fielding, 2019). The N protein is the phosphoprotein that only binds to the RNA genome and involved in the formation of nucleocapsids (Dutta et al., 2020; Zeng et al., 2020; Gao et al., 2021). Understanding the entry mechanism of the virus is very important in the development of new treatments against its infectivity and pathogenesis. Therefore, it is necessary to investigate the functions of the main structural proteins and their effects on the entry mechanism of SARS-CoV-2 (Figure 3) in detail (Al Adem et al., 2020).

The life cycle of the virus begins with its entry into host cells. The SARS-CoV-2 attaches to the host cell surface protein angiotensin-converting enzyme 2 receptor (ACE2) which is the target receptor of SARS-CoV-2 (Coperchini et al., 2020). After membrane fusion, the virion can release RNA into the host. The translation of the structural proteins (except N protein) occurs in the ER due to post-translational modification, while only the translation of the N protein occurs in the cytoplasm. The newly assembled and matured virions are released from the host cell in three different ways by budding, exocytosis, or cell death. The released virions are ready to infect healthy cells *via* reported modes of transmission (Poduri et al., 2020; Chen et al., 2021; Khade et al., 2021; Pizzato et al., 2022).

1.2 COVID-19

The sources of infection and transmission are very important for developing new approaches and controlling the prevention of infection. Normally, the source of COVID-19 is unclear. However, it is believed that the first source may be bats (Yin and Wunderink, 2018; Rahman et al., 2020). It should be noted that, as with bats, other animals sold in Wuhan animal markets, such as civet cats,

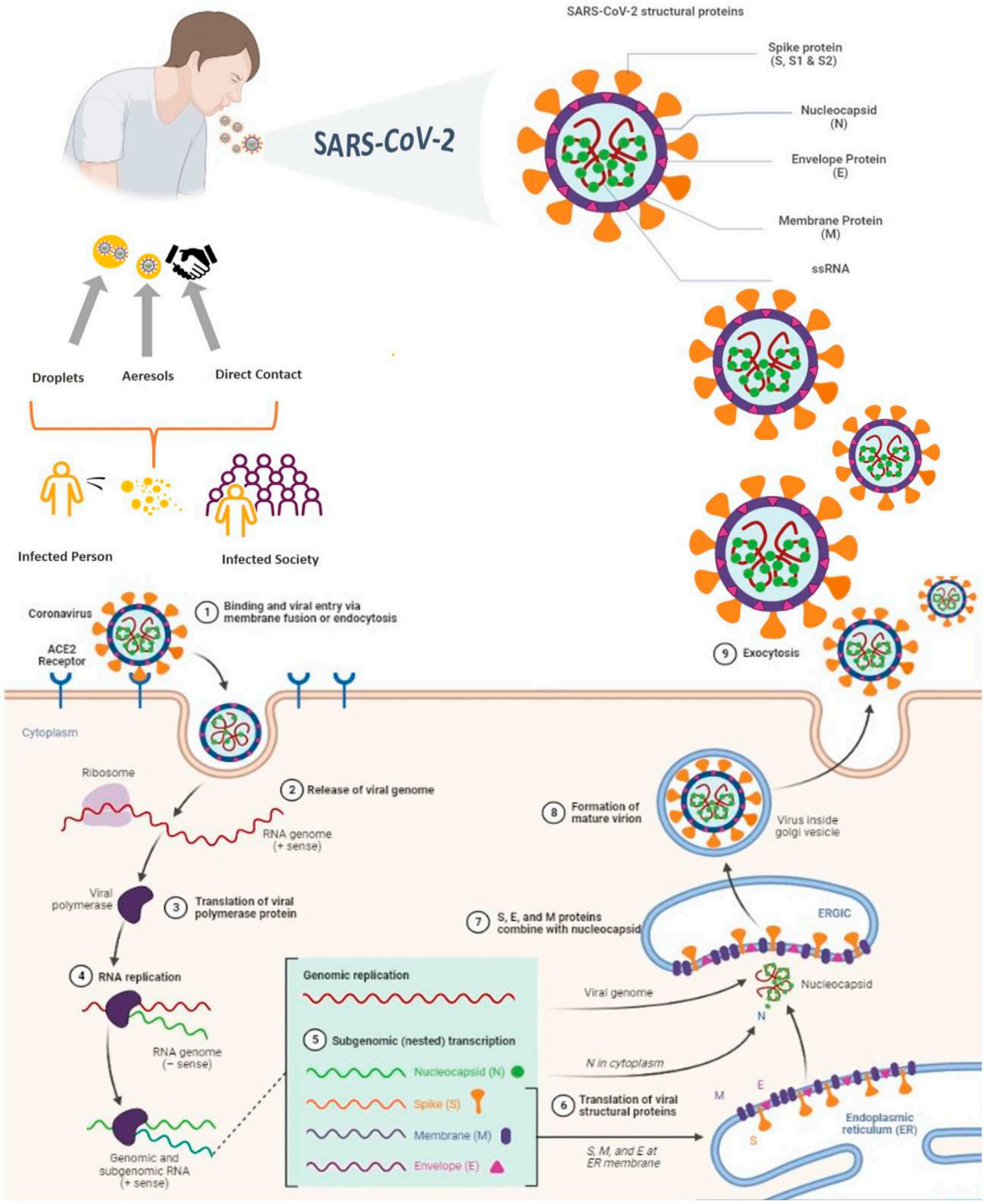

FIGURE 3
Transmission modes COVID-19, life cycle and structure of SARS-CoV-2.

foxes, mink, and raccoon dogs, also carry SARS-CoV-2. When the samples taken from the patients who had contact with these animals and the samples from these animals are compared, it is observed that the virus types are the same. As a result, live animals susceptible to SARS-related CoV were identified as the main progenitors of COVID-19 (Lytras et al., 2021). For this reason, it is even believed that SARS-CoV-2, like other coronaviruses, is transmitted zoonically. However, COVID-19 can be directly transmitted from human to human (Jayaweera et al., 2020).

According to the statements of Chinese health authorities, the transmission in humans occurred *via* direct contact, aerosol, and droplet (Figure 3). Although direct transmission means direct contact

with virus infected objects or surfaces, especially close contact with the virus infected people's mucous membranes of the mouth, eyes, and tears (Crawford et al., 2022). In aerosol or droplet transmission, the diameter of droplet is a critical issue. According to WHO, the droplets have 5–10 μm diameter and the aerosols have less than 5 μm diameter. During respiratory activities such as coughing, breathing, laughing, or talking, both of these large and small droplets can be produced. The viral particles encapsulated within these droplets. Therefore, droplet transmission requires close contact between an infected person and a susceptible person (Ge et al., 2020; Wilson et al., 2020). Also, studies have shown that aerosols can travel more than six feet due to their ability to be suspended in the air (Kutter et al., 2018). Small aerosols are trapped deep in the lungs and cause infection in the lower respiratory tract, while large droplets are trapped in the upper respiratory tract (Jayaweera et al., 2020). According to data from patients, older adults have a higher risk of contracting the disease and a higher mortality rate, because, the levels of hormones in the immune system change during aging. In older adults, some comorbidities such as cardiovascular diseases, respiratory diseases, cancer, and diabetes problems become more prominent, making the elderly more susceptible to COVID-19 (Farshbafnadi et al., 2021). Also, severe COVID-19 is not limited to the elderly population as stated; children and young adults are also at risk. It is inevitable that patients with underlying diseases will experience dangerous symptoms and life-threatening complications if they are infected with the SARS-CoV-2 (Chao et al., 2020; Harrison et al., 2020).

1.2.1 Symptoms

The period between exposure to the virus and the onset of clinical symptoms is called the incubation period, and it is very important in determining the case definition and the establishment of public health programs aimed at taking new precautions against COVID-19 and reducing local transmission. According to WHO, the incubation period of SARS-CoV-2 ranged from 1 to 14 days (WHO, 2020a; Elias et al., 2021). Patients exposed to COVID-19 exhibit mild to moderate symptoms such as headache, fever, fatigue, dry cough, loss of taste and smell, diarrhea, dyspnea, sore throat, chest pain, muscle pain, and abdominal pain (Weng et al., 2021). The clinical features of patients infected with SARS-CoV-2 were first determined by Huang C. et al. (2020; Huang W. C. et al., 2020). According to the classification made by the National Health Institutes (NIH), the disease has five types. First, they are asymptomatic or presymptomatic patients who have positive tests for COVID-19, but do not show clinical symptoms. Secondly, patients with obvious COVID-19 symptoms such as fever, cough, malaise, headache, but no shortness of breath. Third is that patients with clinical symptoms in the lower respiratory tract. Fourth is severe illness that is determined according to a ratio of partial pressure of arterial oxygen to fraction of inspired oxygen. The last one is that critical patients that have especially multiple organ dysfunction, acute respiratory failure, and septic shock. The acute respiratory syndrome begins after 1 week the onset of symptoms and progresses critically (COVID-19 Treatment Guidelines Panel, 2022).

Here, the current nano-based approaches and developments for the prevention, diagnosis, and treatment of COVID-19 detailed in order to emphasize how nanotechnology can help to control viral infections. Moreover, the developed nanotechnological solutions and the connections of multidisciplinary fields during the COVID-19 pandemic are mentioned in sections.

2 Nanotechnology and personal protective equipment for COVID-19

2.1 Personal protective equipment

Although there are many treatment methods developed against COVID-19, there is still a lack of an effective method. In addition, problems such as vaccine and drug development strategies take time, and faster solutions need to be developed to prevent the spread of the virus. The prevention of SARS-CoV-2 without vaccination or other administrative controls could be achieved by using universal infection control standards such as hand hygiene, environmental sanitation, maintaining social distance, personal protective equipment (PPE), and quarantine. However, the most visible but limited control standard is the use of PPE. People who use PPE correctly, especially healthcare workers, are largely protected from the virus and the potential harm they can cause both to themselves and to the environment is reduced. The PPE can act as a physical barrier against viral pathogens. The frequently used PPE types are filtering face piece respirator masks (N types, R types and P types), surgical face masks, gowns/apron, gloves, eye shields, goggles, and boots/closed work shoes (Figure 4) (WHO, 2020b). The applied nanotechnological amplifications contribute to the properties of PPE such as UV protection, antimicrobial properties, and fire retardant (Chintagunta et al., 2021). In addition, nanotechnology can provide hydrophobic and comfortable products for fighting with COVID-19 (Campos et al., 2020).

2.1.1 Nanotechnology in masks

Masks are the most important equipment designed to prevent the entry of pathogens such as viruses from the mouth and nose and to protect both the wearer and the immediate environment from respirable harmful agents. The masks are divided into 3 classes according to their filtering types; single-use face masks, respirator masks, and surgical masks (Figure 5) (Tcharkhtchi et al., 2021).

An ideal mask filters bioaeresols effectively and offers a high level of comfort. However, some external factors like humidity, temperature and pattern of airflow and material properties of masks affect filtration efficiency and mask quality. During pandemic challenge, nanomaterials are the best developed solutions to improve the filtration efficiency of masks when added to textile products. Simultaneously, they can have direct impact on the survivability of viruses that come into contact with the mask. For example, both nanofibers and NPs introduced to masks for improving of antiviral properties, filtering capacity, and breathability properties. Nanofibers produced by electrospinning technology are very useful materials with average fiber diameters in the nanometer range because of their higher surface area to volume ratio that can be easily functionalized with desired property (Ullah et al., 2020). Microorganisms reproduce rapidly in a moist environment. For this reason, thermal optimization of the masks is crucial. In one example, Yang et al. gave a cooling effect and excellent particle filtering properties to masks by using nanofiber on nanoporous polyethylene (Yang et al., 2017).

Although NPs have many advantages, high surface/volume ratio causes an increase in toxicology. They carry many risks for both public health and the environment due to insufficient epidemiological studies (Valdiglesias and Laffon, 2020). Priority should be given to the development of new systems that can test the effects of nanoparticles on human health and the environment so that they can be applied to masks (Palmieri et al., 2021). As one of the new

FIGURE 4
PPE used in the COVID-19 pandemic.

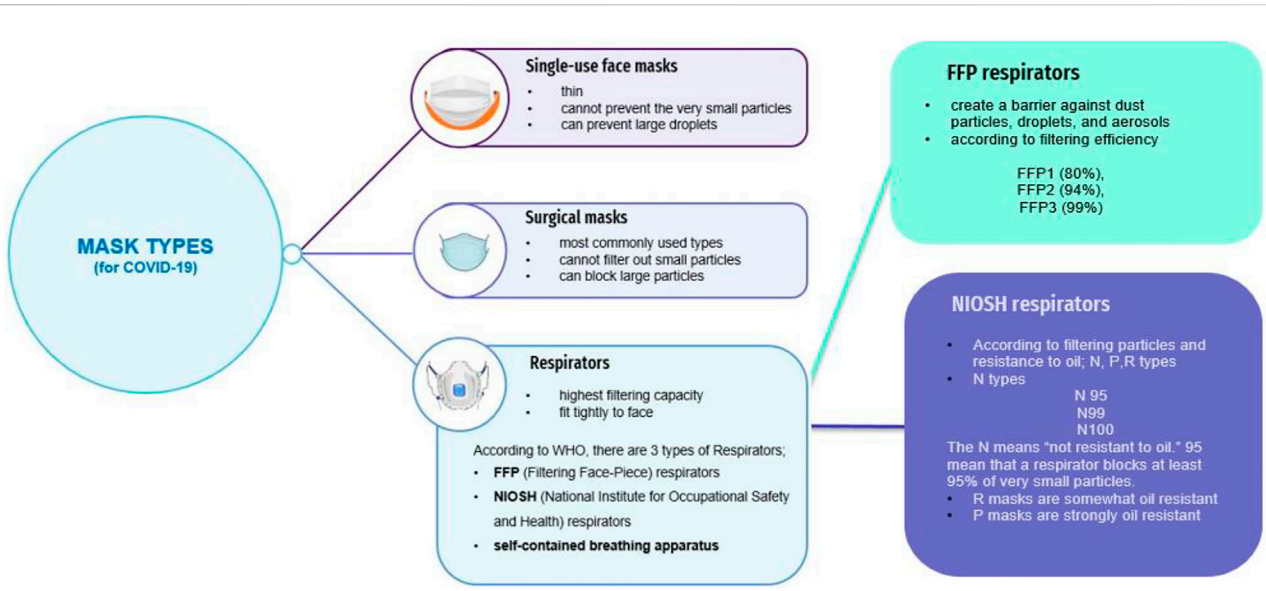

FIGURE 5
Mask and respirator types for COVID-19.

technologies, three dimensionally (3D) printing technology was applied to produce face masks against COVID-19. The new masks were designed with reusable purposes. In 3D-printed masks, only filter material requires consistent replacement. The other parts can be easily recycled and therefore 3D-printed masks are more sustainable than single-use face masks (Choong et al., 2020; Deng et al., 2022). According to new studies, Swennen et al. (Swennen et al., 2020) have produced 3D printed, custom-made, reusable N95, personalized masks. They used two reusable polyamide composite components (face mask and a filter membrane support) and two disposable components (a head fixation band and a filter membrane) during production. Although 3D face masks combined with FFP2/3 filter membranes are presented as a valid alternative source, there is no virological validation and no data on the safe reuse of the face mask with new filter membranes and headbands. In another study, a 3D printed mask was produced by Provenzano et al. (Provenzano et al.,

2020). These simple materials are composed of reusable and printable headpiece to which different transparent plastic sheet can be connected to form a face shield. They can easily protect user's eyes and mouth (Tino et al., 2020).

Although 3D-printed masks provide some protection and are more sustainable, their filtering performance has not yet been approved by any regulatory agency. Filtering efficiencies are very low due to leakages between the interfaces on the printed mask. Therefore, it is not recommended to use 3D-printed masks as an alternative to medical masks until sufficiently sensitive and reliable tests are developed to measure filtration efficiencies. In addition, the printing parameters should be improved, and further optimizations should be made by the researchers for increasing the filtration efficiency (Deng et al., 2022).

2.1.2 Nanotechnology in gloves

Medical gloves are one of the important PPE used by healthcare professionals to prevent the spread of the virus. There are several types of gloves (Tabary et al., 2021). However, nitrile and latex-based gloves have been used frequently in the pandemic. The biggest problem of gloves is the inappropriate use of them. If they are used uncontrollably, they can prepare a shelter environment for viruses and microorganisms. Especially, if hands are touched to the face after contact with unclean surfaces, the risk of virus transmission is very high. Therefore, with the addition of virucidal agents, the risk of virus transmission of gloves is reduced. As with masks, many studies have been carried out using nanotechnology in gloves. The antibacterial effects of silver nanoparticles (AgNPs) were used in glove production and it was proven to have virucidal activity (Aydemir and Ulusu, 2020). In another aspect, viruses can enter host cells by using the ACE2 receptor. If the ACE2 level of the body is decreased, the penetration of the viruses can be blocked and reduced. Therefore, the catching of the viruses before entry into cells by using nanotechnology would be exceedingly helpful. As a result of the studies, it has been observed that nanomaterials containing ACE2 reduce the infection rate. ACE2 proteins coated with NPs neutralize viruses and prevent them from entering host cells. It is extremely important to use nanotechnology-based, nanoparticle coated ACE2 proteins in the production of glove (Aydemir and Ulusu, 2020; Rasmi et al., 2021). However, without relying solely on nanotechnology, care must be taken to use gloves carefully (Yadav D. K. et al., 2020).

2.1.3 Nanotechnology in disinfectants

Disinfectants or antiseptics are chemical substances specially prepared to inactivate or destroy microorganisms. If it completely kills microorganisms in the places where they are used, sterilization is achieved. However, the effectiveness of a disinfectant depends on its chemical composition, the characteristics of the pathogen in which it is used, and the intracellular vulnerability. One of the most important ways to prevent COVID-19 is to reduce transmission routes by using disinfectants. The disinfectant types that are frequently used in the COVID-19 are classified as alcohol, phenol, formaldehyde and glutaraldehyde-based, oxidizing agents, chlorine, and iodine releasing agents. The mechanism of each disinfectant is different. For example, alcohols denature proteins in microorganisms and cause cell lysis by causing membrane damage. In particular, it was stated that ethanol showed virucidal activity on both living and non-living surfaces (Al-Sayah, 2020; Dhama et al., 2021). Another agent, chlorine, damages the cell wall of microbes by affecting the oxidation of lipids and proteins due to its electronegativity. If the lipid E protein, S protein, glycoproteins, and viral genome of the virus are damaged or disrupted, the virus loses its activation and infectivity (Al-Sayah, 2020).

Considering that the virus is likely to be transmitted to many surfaces during the pandemic, frequent disinfection and sanitization of hands, touched objects and surfaces are very important. However, the possible effects of constantly used disinfectants on humans, animals, the environment, and ecological balance should not be ignored. Therefore, the development of safer and more environmentally friendly disinfectants as an alternative will help reduce the side effects of chemical disinfectants. Nanotechnology enables the production of safer and healthier products in this regard. In particular, metallic NPs such as silver, copper, and titanium have been shown to have virucidal activity against SARS-CoV-2. According to the studies, multifunctional disinfectants were obtained by adding AgNPs to sanitizers using nanocolloidal techniques. The disinfectant obtained has a broad spectrum as it shows activity against viruses, bacteria, and fungi. The silver ions in the disinfectant inactivate the protein structures of microorganisms by denaturing them (Rasmi et al., 2021). Another significant topic is the size of the particles. It has been reported that NPs smaller than 20 nm can easily bind to pathogens including SARS-CoV-2 and cause death (Campos et al., 2020). NanoTech Surface Company, has developed a nanopolymer-based (containing silver and titanium dioxide NPs) disinfectant for disinfection of coronavirus-infected surfaces. The most important advantages of the developed disinfectant were that it was eco-friendly, non-flammable, and biodegradable (StatNano, 2022). In another study, nano-disinfectants that can neutralize microbes both in the air and on surfaces have been developed. The developed nano-disinfectant consists of various components such as deionized water, electrolyzed water, and hydrogen peroxide can be used in COVID-19 (Vaze et al., 2019).

3 Using nanoparticles in COVID-19 testing

Nanotechnologies integrated into NPs demonstrates that nanomaterial-based approaches can lead to an important improvement in NP target detection performance (Medhi et al., 2020; Chowdhury et al., 2021). Among the presented nanotechnology approaches is the use of NPs in personalized medicine applications that do not require complex or expensive equipment for signal detection (Abdellatif and Alsowinea, 2021). In addition, the ability to measure the test reading is important for devices used in personalized medicine applications. Therefore, integrated nanotechnologies in NPs should allow easy signal quantification, either semi- or full-quantitatively, according to the type of biomarker being studied (Izcovich et al., 2020). The early diagnosis of viral is critical to reduce the possibility of widespread outbreaks in hospitals and nearby populations. Normally, the diagnosis strategies can be investigated in molecular tests, serologic tests, and imaging techniques (Cascella et al., 2020). The first step of diagnosis is based on the history of patients. The second step is recommendation of Chest-X-Ray and CT (Computed Tomography) to detect glass opacity and patchy bilateral shadows in the lungs (Tavakol et al., 2021). The molecular identification of the SARS-CoV-

2 genome in pharyngeal swab samples using a real-time back transcription-polymerase chain effect (rRT-PCR) test is the gold standard for COVID-19 diagnosis. rRT-PCR used to detect the RNA genome of SARS-CoV-2, however, it requires biosafety level 2 or above (Corman et al., 2020) and results of it are obtained for up to 3 days. Therefore, it is necessary to develop new point-of-care (POC), cost-effective, fast, and highly reliable devices. The serological tests for specific immunoglobulin -M (IgM) and -G (IgG) has been offered as an alternative method to decrease false negatives rate related with rRT-PCR test but the results showed that they have limited benefit (Latiano et al., 2021). Screening of symptomatic and asymptomatic patients is different. Upper respiratory tract specimens such as nasopharyngeal swabs or nasal aspirates are used to screen asymptomatic patients, while lower respiratory tract specimens such as sputum or bronchoalveolar lavage are used in symptomatic patients. In addition, saliva is also used to detect respiratory viruses. The most important advantage of using saliva is that it reduces waiting time for patients and reduces the risk of viral transmission to healthcare workers. It has also been reported that the rate of detecting respiratory viruses in saliva is comparable to Nasopharyngeal swabs (Ochani et al., 2021). All of the diagnosis tests and imaging procedures have their advantages and disadvantages. However, nanotechnology is the best tool to use in the development of new tests for detecting SARS-CoV-2 (Tharayil et al., 2021).

Biosensor technology plays a significant role in diagnosing COVID-19 (Behera et al., 2020; Choi, 2020; Leïchlé et al., 2020; Samson et al., 2020; Abid et al., 2021; E, S, Innovation Center et al., 2021; Maddali et al., 2021), because of their cost-effectiveness, high sensitivity, biocompatibility, and mass production potential (Behera et al., 2020). Biosensor is a device containing biological sensing material combined with a physicochemical transducer that can convert a biological signal into a measurable and processable electrical signal (Abid et al., 2021). Currently, various types of biosensors have great commercialization potential to meet the disadvantages by the COVID-19 (Fathi-Hafshejani et al., 2021). With the development of biosensor technology, it can be said that a new era has begun in disease diagnosis. In fact, it will be possible to keep the patient under constant observation thanks to the biosensors placed inside the body (Bahl et al., 2020). Here, biosensors, their working principles, importance technologies for COVID-19, and primary application in medicine briefly discussed.

3.1 Components and working principle of biosensors

The block diagram of the biosensor consists of three parts: sensor, transducer, and analyte. If the receptor is in a biomolecular structure, it is called a bio-receptor. Bio-receptors are biomolecules (enzymes, tissues, nucleic acids, antibodies, receptors, and organelles) that can recognize the analyte (Behera et al., 2020). Bio-receptors with the suitable combination transform this analyte with the analyte-specific bio-receptor structure (Behera et al., 2020; Al-Douri et al., 2021; Wu and Wu, 2021; Yasri and Wiwanitkit, 2022). Transducers, on the other hand, are formations that transform the neither chemical nor physical signal manufactured by the bio-receptor when recognizing the analyte into electrical signals (Behera et al., 2020). In biosensors; bio-receptor materials send detectable signals to the transducer which can be used in the system as piezoelectric, optical, electrical, electrochemical, thermal, and others. The bio-receptor and the transducer are associated to each other by an appropriate neither physical nor chemical pathway (Behera et al., 2020; Al-Douri et al., 2021; Wu and Wu, 2021; Yasri and Wiwanitkit, 2022).

The transducer is one of the most important element of biosensor (Zhao et al., 2021a). The moderately deliberate atmosphere of the biosensor reaction clearly relieves electrical noise purification anxiety (Pang et al., 2021). An analog wave or a signal is converted in a digital arrangement and recognized to a microprocessor progressive output. Here, information is processed, tailored to preferred units, and sent to data storage. Physical type biosensor applications are able to offer a direct advantageous effect in tackling challanges arising from the COVID-19 (Ashraf et al., 2021).

3.2 Biosensor technologies and COVID-19

Biosensor technologies have an essential role in diagnosing COVID-19 (Choi, 2020). In the last 20 years, the biosensor-based instruments have been accelerated in medical diagnostics, because of their biocompatibility, cost-effectiveness, high sensitivity, and potential for mass production (Bahl et al., 2020; Xu and Li, 2020; Mobed and Sepehri Shafigh, 2021; Zare et al., 2021). Currently, various types of biosensors have great commercialization potential to meet the challenges posed by the COVID-19 (Murugan et al., 2020; Fani et al., 2021; Saki et al., 2021). Certainly, these sensors have enormous potential in other fields, containing medical diagnostics, and warrant further research (More, 2021; Narita et al., 2021; Wu and Wu, 2021).

Industry 4.0's digital technologies have the ability to detect COVID-19 symptoms (Wu and Wu, 2021). This helps to avoid confusion and predict the probability of contracting disease (Sharma et al., 2021). Thermometric and piezoelectric biosensors are types of physical sensors (Bahl et al., 2020). Further sensing is accomplished using NPs, while thermometric biosensors work to measure temperature changes associated with biological samples (Wu and Wu, 2021). Thermal biosensors are generally used to measure cholesterol (Dinnes et al., 2021). These biosensors can be easily worn on a daily basis and can be used with everyday clothing; t-shirts, pants, headphones, and wrist watches. Therefore, thanks to its easy use, the real-time health status of the patient can be checked (Kudr et al., 2021).

Various types of biosensors are present to supply medical care benefits to humans (Abid et al., 2021; Wu and Wu, 2021). Electrochemical, optical, physical, and wearable biosensors are the main categories of biosensors (Ribeiro et al., 2020; Shrivastav et al., 2021). Researchers combined nanotechnology with traditional optical biosensor technology (Murugan et al., 2020; Nag et al., 2020) to successfully detect SARS-CoV-2. A new nanotechnology-based biosensor named 'Graphene-Field-Effect Transistor biosensors' (Xu S. et al., 2021) can detect changes in the environment on their surface and provide ultra-sensitive and low-noise sensing. However, the performance of the developed nanotechnology-based biosensor is specified using antigen protein, cultured virus, and nasopharyngeal swab samples from COVID-19 patients (Vashist et al., 2012; Maheshwari et al., 2021). Nanocomposites based biosensors are able to be used to monitor and re-transport infections in food for COVID-19 patients. Nanomaterials increase their adaptability and sensing abilities

(Huang et al., 2021). A newly released biosensor technology has been developed for COVID-19 that illustrated a high value of specificity and sensitivity (Lizhou Xu and Shoaie, 2020). This optical biosensor contains a gold nanostructure on a glass substrate. In the future, it is foreseen to develop a biosensor that can be used in busy places to determine the presence of coronavirus in the environment and measure its concentration in the air in real time, and the recorded data will be sent wirelessly and monitored in real time (Shand et al., 2022).

3.3 Lateral flow tests and COVID-19

Various types of biosensors have been advanced to determine biomarkers linked with various diseases (Gupta et al., 2020; Kim et al., 2021; Pérez-García et al., 2021). Lateral flow tests (LFTs) (Cui and Zhou, 2020; Rosati et al., 2021), which are paper-based devices, are able to meet the needs wait of a biosensor (specific identification of target analyte, low cost, stability, user-friendly test format, fast, and low sample volume) (Ozturk et al., 2021). In addition, proteins, nucleic acids, and whole cells are biomarkers of LFTs, and they serve as POC testing (Mlcochova et al., 2020). This can provide support for patients and personalized medicine. Paper-based POC immunoassays, particularly LFTs, have received great attention because of the requirement for joint qualitative and quantitative biosensing applications. For the purpose of diagnosing human health, different LFTs have been commercialized for the detection of different markers (Violan et al., 2021). Nanomaterials have been used for the development of POC diagnostics and delivery strategies. Despite the numerous benefits of LFTs, they have some challenges, such as difficulty of sensitivity detection of a particular target analyte without applying signal amplification strategies (Rodriguez-Manzano et al., 2020). Availability of cost-effective rapid diagnostic testing is very important for physicians in emergency departments, clinics and hospital. These diagnoses allow nurses and doctors staff to simply prioritize patients, and hinder further spread of disease (Uzay and Dinçer, 2022).

3.3.1 Observation and monitoring

Rapid identification of the disease is one of the factors preventing the spread of a mass infection. The success of a system depends on effective collaboration and communication between federal and state public health laboratories, hospitals, government agencies, and communities. WHO testing should be widely used to stop this epidemic (Bonnechère et al., 2021).

3.3.2 Therapeutics

Once individuals with COVID-19 are identified, patients need to be treated. These treatments can inhibit the replication of SARS-CoV-2 in the host. Fundamental studies of nano-bio interactions are about explaining how SARS-CoV-2 infects its cells (Singhera et al., 2021). Nucleic acid amplification tests (NAATs), such as polymerase chain reaction (PCR), primarily detect viral genomic RNA encoding S and N proteins (Kamat et al., 2021). Antigen tests, such as LFTs, primarily detect the N protein. NAATs are the gold standard diagnostic for SARS-CoV-2 infection (Tyagi et al., 2020). SARS-CoV-2 has developed many mutations that can result in immune escape (Moabelo et al., 2021). Since NAATs use nucleic acid primers to recognize and amplify their targets, the incorporation of one or more base substitutions into the targeted nucleotide sequence is potentially sufficient to inhibit the reaction. LFTs are inexpensive, user-friendly and rapid diagnosis that can be used at the POC (Jain et al., 2020).

In comparison with the S protein, the N protein is relatively conserved from variant to variant (Devi et al., 2021; Weinberg et al., 2021). Consequently, detection of N protein instead of the S protein should result in a test that is more resistant to diagnostic evasion. In fact, every rapid detection test approved by the FDA under Emergency Use Authorization since 2020 is still recommended for SARS-CoV-2 detection (Tinberg et al., 2013). Companies have developed rapid tests using mAbs previously isolated from SARS-CoV N protein immunizations (Gage et al., 2021) However, thanks to continuous accumulation of mutations in the N protein may be used to maintain high diagnostic efficiency for improved mAbs and diagnostic tests specifically for SARS-CoV-2 (Tinberg et al., 2013; Sandersjöö et al., 2015; Nilsson et al., 2017; Yadav N. et al., 2020; Gage et al., 2021). Prototype LFTs are available for detection of SARS-CoV-2 N protein in clinical samples (Jain et al., 2020; Dowlatshahi and Abdekhodaie, 2021).

4 Developing a vaccine against COVID-19

Vaccines are very special and an essential component of public health, as they protect individuals and communities (Sebastian et al., 2020; Excler et al., 2021). Edward Jenner made the first successful vaccine study in the late 1700s. To date, vaccine development and large-scale immunization campaigns have been used by society against worldwide epidemics of infectious diseases (Verbeke et al., 2021). There are some criteria for developing an ideal vaccine. The developed vaccine must be safe and highly effective, even in immunocompromised individuals. In addition, it should be inexpensive, free from toxicity/side effects, capable of long-term protection, and high thermal stability (Malik et al., 2021).

The development of a new vaccine is a complicated process that takes 10–15 years (Sebastian et al., 2020). The economic situation of the countries has a very important role in the creation of a new sustainable vaccine development model, in which significant investments make and which includes close cooperation between the public and private sectors. In low- and middle-income countries, most vaccines for diseases cannot be developed due to weak or lacking market incentives (Rappuoli et al., 2019). The process of the development of vaccines is mainly based on four different stages: discovery, pre-clinical development, clinical development (Phase I, Phase II, and Phase III), and post-marketing. However, unlike traditional vaccine development processes, it has enabled the rapid development of COVID-19 vaccines based on the knowledge available in vaccines developed for COVID-19 (Figure 6) (Ndwandwe and Wiysonge, 2021).

In a fast-paced research environment such as the COVID-19 pandemic, using samples collected in December 2019, and then Edward C. Holmes et al. published the full genome sequence of SARS-CoV-2 on virological.org on 10 January 2020. On 11 January 2020, vaccines against COVID-19 began to be developed, when the genome sequence of SARS-CoV-2 made available by the United States (US) NIH (Haque and Pant, 2020; Wang C. et al., 2020). The COVID-19 vaccines currently approved for use in the US are Pfizer/BioNTech, Moderna, Novavax, and Johnson & Johnson's Janssen (CDCP, 2022). In the United Kingdom Moderna, Oxford/AstraZeneca, and Pfizer/BioNTech vaccines currently approved (National Health Service, 2022). In Türkiye vaccination first started with the inactivated

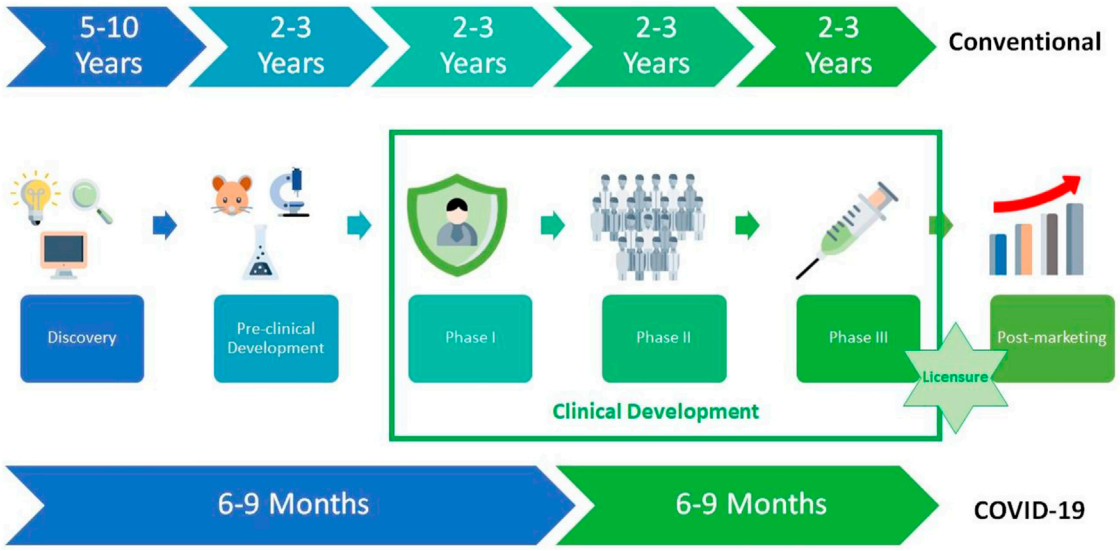

FIGURE 6
The process of the development of vaccine for both conventional and COVID-19.

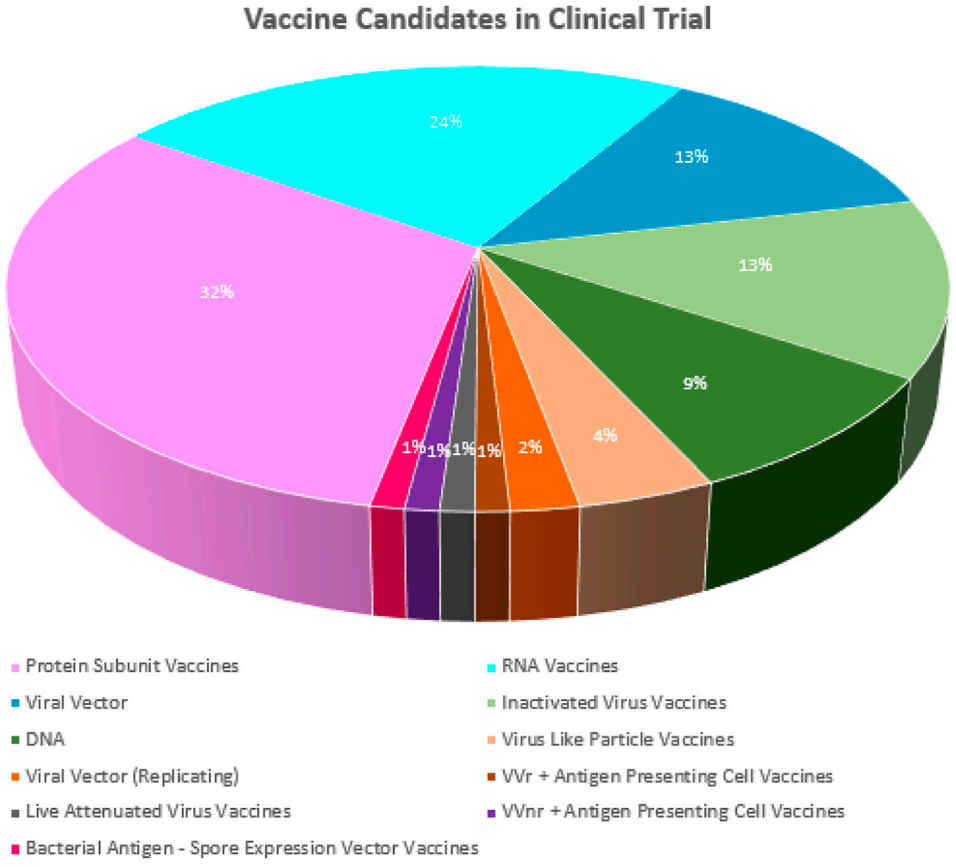

FIGURE 7
Current stage of development of clinical 11 November 2022 (Recreated from WHO (WHO, 2022b).

CoronaVac, then with the mRNA BNT162b2 vaccine (Batibay et al., 2022). According to the WHO COVID-19 Dashboard on 19 September 2022, a total of 12.640.866.343 vaccine doses were administered (WHO, 2022b).

4.1 The types of vaccines for COVID-19

Adjuvants plays important role for enhancing vaccine efficacy (Lee et al., 2022). Rino Rappuoli and colleagues introduced the genome-based reverse vaccination method with strong antibody responses. However, investigation of other factors (i.e. adjuvants and antigen delivery), has gained momentum as they influence the induction of immune responses and immunological memory, which are important for vaccine efficacy (Raeven et al., 2019). Alum, complete Freund's adjuvant, thiomersal, incomplete Freund's adjuvant, montanide, lipovant, and adjuvant 65 are currently available adjuvants used to develop a subunit vaccine (Nevagi et al., 2019). In addition to finding new adjuvants, researchers are also modifying existing adjuvants to increase immune-inducing ability and reduce toxicity. It is known that their modification of the epitope further enhances the immunogenicity of subunit vaccines, increasing stability, half-life, and solubility which collectively increase the Th1 response (Mekonnen et al., 2022).

Multiple methodologies have been adopted for the development of vaccines against SARS-CoV-2, including both next-generation and conventional techniques (Malik et al., 2021). Researchers around the world have been racing to develop COVID-19 vaccines, with more than 175 candidate vaccines in the clinical evaluation stage and another 199 vaccines in preclinical evaluation as of 11 November 2022 (WHO, 2022b) (Figure 7). Nowadays, more than 150 companies or educational institutions offer are making efforts to combat the current coronavirus pandemic with strategies (Malik et al., 2021) such as live attenuated vaccines, inactivated vaccines, subunit vaccines, vector based vaccines, DNA-based vaccines, and mRNA-based vaccines.

4.1.1 Live attenuated vaccines (LAV)

Chemically inactivated and genetically engineered vaccines have been developed since 1896, and up to 15 LAVs (against 12 viral and 3 bacterial diseases) have been used (Pöyhönen et al., 2019). Composed of live but attenuated microorganisms that aim to activate innate immune responses, LAVs do not cause disease in humans but also contain replication-competent viral vectors. LAVs can induce prolonged, robust cellular and humoral immune responses without the need for adjuvants (Mok and Chan, 2020). LAVs can be much more effective compared to other vaccines as they are similar to excellent mRNA vaccines or vector vaccines and can unparalleled manufacture multiple, endogenous and relatively large amounts of viral antigens. However, LAVs are not suitable for viruses that reproduce poorly in *in vitro* analysis (Chen, 2022). The bacille Calmette-Guerin (BCG) vaccine has been administered for the prevention of *tuberculosis* (Brooks et al., 2021). However, BCG can lead to heterologous immunity by the mechanism of stimulation of antigen-independent B and T Cells, resulting in reduced viral load of SARS-CoV-2 (Khera et al., 2021).

4.1.2 Inactivated vaccine

Purified inactivated vaccines used in conventional vaccine development have been found to be safe and effective in preventing diseases caused by pathogens such as influenza virus and polio virus (Gao et al., 2020). CoronaVac, which is an inactivated whole virus vaccine adjuvanted with aluminum hydroxide and developed by Sinovac Life Sciences (Beijing, China) for COVID-19, was one of the first vaccines to be distributed globally (Wilder-Smith and Mulholland, 2021; Wu Z. et al., 2021). PiCoVacc (Gao et al., 2020), Covaxin (BBV152) (Ahmed et al., 2022), and CoviVac (Kozlovskaya et al., 2021) are other candidate inactivated SARS-CoV-2 vaccines.

4.1.3 Subunit vaccine

Subunit vaccines show a new generation of vaccines that use pathogenic components (antigens) in parasites, bacteria, or viruses to stimulate adaptive immunity against them. Recently, protein and peptide antigens are in use worldwide (Malik et al., 2021). Characteristic subunit vaccines are built on antigens that are much safer and highly purified than whole organism-based preparations. In these vaccines, adjuvants are often included in the vaccine formulation to improve an immune response. One of the biggest disadvantages for subunit vaccine is developing adjuvants with no or minimal toxicity, as currently available adjuvants often lead to toxicity and reactogenicity (Nevagi et al., 2019). In the literature, the S1 domain and its RBD have been shown to induce much higher IgG and IgA antibody levels when immunized in mice and neutralize SARS-CoV-2 more efficiently when adjuvanted with alum. However, it is stated that Th1 response-prone adjuvants should be used for S1-based subunit COVID-19 vaccines to reduce the potential risk of increased antibody-induced infection (Wang et al., 2021). StriFK (Wu Y. et al., 2021), S-Trimer (Liang et al., 2021), ZF 2001 (Yang et al., 2021), and Novavax (Callaway and Mallapaty, 2021) are other types of subunit vaccine candidates.

4.1.4 DNA vaccines

DNA vaccines are vaccines that produce antigens by causing a protective immunological response and is able to stimulate a wide variety of immune responses, and the antigen encoded by the DNA vaccine is injected into cells by introducing an adjuvant that induces a concerted immune response (Duman et al., 2021). Inovio Pharmaceuticals has already developed experimental vaccines (INO-4700) against COVID-19 (Smith et al., 2020). The S protein is used as the antigen of all DNA vaccines currently being tested in clinical trials (Silveira et al., 2021). However, since 2016, DNA vaccines have not been released in the US (Duman et al., 2021).

4.1.5 Vector based vaccines

The viral vector acts as a delivery system, providing a means to attack the cell and insert the code for SARS-CoV-2 antigens that it does not cause illness by being chemically weakened. In this way, the body is able to be safely mount an immune response without contracting the disease (Ndwandwe and Wiysonge, 2021). Johnson & Johnson's Janssen vaccine (Yang et al., 2022) and Sputnik V vaccines are vector based vaccines. Gam-COVID-Vac (Sputnik V), a heterologous recombinant adenovirus-based vaccine, is known to show a good safety profile in participants in phase 1/2 clinical trials and induce potent humoral and cellular immune responses (Logunov et al., 2021).

4.1.6 mRNA vaccines

Production of mRNA can be accomplished in a one-step enzymatic reaction using a capping analog, or in a two-step reaction in which closure is accomplished using a vaccine capping enzyme. Purification on a larger scale is achieved using well-established chromatographic strategies (Rosa et al., 2021). Moderna (mRNA-1273) and Pfizer-BioNTech (BNT162b2) have taken into clinical trials at an unprecedented rate in less than 1 month, obtaining clinical use authorization within 1 year, breaking the latest 4-year speed record held by the mumps vaccine (Ziqi et al., 2022), and CVnCoV, moved into Phase III clinical testing as a mRNA vaccine candidate (Verbeke et al., 2021).

4.2 Nanotechnology and COVID-19 vaccines

Nanotechnology allows the development of nanoscale systems derived from interactions between surfaces and biomolecules. The most obvious approach to nano-sized vaccines is based on VLPs, which can achieve enhanced safety (compared to all virus-based vaccines), a marked immunogenicity (relative to soluble antigens) due to their complexity, and potent and protective immune responses (Palestino et al., 2020). The role of nanotechnology in COVID-19 is crucial for designing effective nanocarriers to counter the traditional limitations of nanointervention, antiviral, and biological therapeutics (Chauhan et al., 2020). Unlike inactivated or killed virus vaccines, mRNA and other bioengineered vaccines can be quickly and inexpensively modified to match mutated antigenic epitopes (Shapiro, 2021). Due to the presence of RNA-degrading enzymes and their inability to easily pass through the negatively charged cell membrane, LNP-based delivery systems have been designed to maintain mRNA integrity and promote its intracellular uptake (Khurana et al., 2021). Moderna vaccine formulation contains lipid nanoparticle-encapsulated mRNA that encode S protein (Thanh Le et al., 2020). The use of engineered nanocarriers to control and eradicate the spread and recurrence of this pandemic has the potential to require a safe and effective vaccine strategy (Chauhan et al., 2020). It is significant to the rapid approval of two mRNA vaccines for nanotechnology's common use in COVID-19, including protein NPs (for the delivery of protein vaccines), lipid NPs (for formulation with mRNAs), and nanobodies (as unique therapeutic antibodies) (Du et al., 2022). NPs are considered as carriers to which multiple ACE2-based peptide inhibitors are attached to their surface. It is important to design NPs vaccine carriers with greater protection, durability, immunization and greater accessibility to target cells of the COVID-19 vaccine (Jafari et al., 2022).

5 Nanomaterial-based drug delivery systems

At the beginning of the COVID-19, treatment options were quite limited. For this reason, many researchers, including clinical researchers, have made continuous efforts to develop new therapeutics and vaccines. In the initial course of the disease, antiviral agents, inflammation inhibitors, and hyperimmune immunoglobulins were used. While antiviral agents stop the progression of the disease, it has been observed that the use of both antiviral and immunomodulators gives good results in critically infected patients (Cascella et al., 2020). The main problem of antiviral agents is that they cause cell cytotoxicity. In addition, the mutagenic structure of the virus causes loss of efficacy of traditional therapeutics. However, if the virus mutates, they lose their effectiveness. Therefore, the development of new antiviral-based materials is critical. Nanotechnology offers interesting and valuable solutions in this regard. Therefore, existing approaches should be advanced based on nanotechnology (Campos et al., 2020).

Nanotechnology has wide applications in the treatment of COVID-19, and with its potential to inhibit virus-cell interaction, transcription, membrane fusion, cell internalization, translation, and viral replication as well as activating intracellular mechanisms (Mainardes and Diedrich, 2020). Due to composition, luminescence, shape, huge surface-to-volume ratio, tunable size, and the ability to reveal multiple interaction sites on the surface, inorganic NPs (INPs) such as mesoporous silica NPs (MSNPs) (Abdelhamid and Badr, 2021; Abdellatif and Alsowinea, 2021; Rasmi et al., 2021), gold NPs (AuNPs) (Miró et al., 2021), iron oxide NPs (IONPs) (Subhash and Chaudhary, 2021), silver nanoparticles (AgNPs) (Douaki et al., 2020). On the other side, organic NPs (polymeric nps, lipid-based NPs, dendrimers, liposomes) are also advantageous because of the possibility of site-specific targeting of drugs, biodegradability, controlling drug release, biocompatibility, and non-toxicity (Abhyankar et al., 2021; Refaat et al., 2021; Thi et al., 2021), and nano micelles (Skwarek et al., 2021).

Biodegradable polymers approved by the FDA and the European Medicines Agency are used in the development of NPs for the delivery of new antiviral molecules. Polymeric nanoparticles are used against viral diseases as also they can be designed to achieve specific targets and inhibit virus binding to host cell receptors (Kamat et al., 2021; Pelosi et al., 2021; Li et al., 2022; Mazayen et al., 2022). It is necessary to improve the safety of antiviral drugs and overcome cellular drug resistance (Rana, 2021). Mesoporous silica NPs, which could supply an excellent platform to treat COVID-19 by preventing viral replication, have pores of adjustable size that allow molecules to dock inside and outside for co-delivery (Karaman et al., 2021; Tiamiyu et al., 2021; Xu L. D. et al., 2021).

Carbon nanotubes (CNTs) exhibit limitations in terms of toxicity for the treatment of COVID-19 (Sadighbayan et al., 2020; Jeong et al., 2021; Joshi et al., 2021). When exposed to the lungs, they activate macrophages in the lower respiratory tract, causing fibrosis and collagen formation in the lesions (Bisht et al., 2021; Galal et al., 2021; Özmen et al., 2021). Lipid-coated MSNPs containing antiviral agent (ML336) were developed as a strategy to improve antiviral circulation time and biocompatibility. *In vitro* results revealed a dose-dependent virus inhibition and an additional release of ML336 after cell endocytosis, while *in vivo* results showed that NPs showed significant antiviral activity and no toxicity (Siddiquie et al., 2020; Balkrishna et al., 2021; Duan et al., 2021).

Dendrimers with compact spherical structure and highly branched 3D structures have unique physicochemical properties such as solubility, low polydispersity, effective drug encapsulation capacity (Jana et al., 2019; Witika et al., 2020; Mazayen et al., 2022), biodegradability, and biocompatibility (Paull et al., 2020; Khaitov et al., 2021). The ability of dendrimers to form strong interactions with viruses may increase antiviral activity, making them promising systems against viral infections (Yuliani, 2021; Zhang D. et al., 2021; Zhang et al., 2022). Composed of synthetic or natural phospholipids,

cholesterol, and sphingolipids, liposomes are bilayer structures used for drug delivery (Abhyankar et al., 2021; Refaat et al., 2021; Thi et al., 2021). Liposome-based carriers are used to encapsulate antivirals for the treatment of infected cells (Huang W. C. et al., 2020; Khurana et al., 2021; Pascolo, 2021; Tenchov et al., 2021; Vahedifard and Chakravarthy, 2021).

5.1 Cell-based therapy and COVID-19

Cell therapies are of great importance in the treatment of severe COVID-19 manifestations because of their personalized and regenerative functions (Shih et al., 2020). While the subacute and chronic sequelae of COVID-19 may be important data, it is vital that all research focuses on finding targeted therapies that not only reduce acute injury but also restore physiological function (Maurya et al., 2020; Shih et al., 2020; USA National Institutes of Health, 2020; Rafat et al., 2021; Vaka et al., 2022). Thanks to ACE2 (Ni et al., 2020), transmembrane protease-serine-2 (TMPRSS2), endosome-proteases-cathepsins B/L (H.-I. et al., 2020; USA National Institutes of Health, 2020) and Neuropilin 1 (NRP1) (Cantuti-Castelvetri et al., 2020; Daly et al., 2020; Abebe et al., 2021) virus can be taken up into the cell. SARS-CoV-2 selects and modifies various cellular proteins and pathways, many of which have not yet been fully clarified (Beyerstedt et al., 2021). Information from other coronaviruses has demonstrated the presence of CD147 and 78 kDa glucose/regulated/protein (Ibrahim et al., 2020; Sabirli et al., 2021) as putative alternative receptors. However, further research is being actively exploring the bulk tissue distribution of these factors correlates with viral tropism as well as illness symptoms (Abassi et al., 2020; Snyder and Johnson, 2020; South et al., 2020; Zhang et al., 2020; Gottschalk et al., 2021; Kragstrup et al., 2021).

6 Future research

Nanomedicine is a growing field that includes the application of nanotechnology to diseases; diagnose-treat-prevent (Tharayil et al., 2021; Yang, 2021). It incorporates a number of disciplines including material science, biology based fields (virology and molecular biology), and more to provide innovative disease management strategies. Many drugs are not specific to their targets, resulting in undesirable side effects and toxicity in patients (Rasmi et al., 2021; Vahedifard and Chakravarthy, 2021). Nanotechnology may allow targeted drug delivery to a specific cell population, as in the novel targeted cancer therapies. In addition, Pfizer and Moderna overcome challenges with the physical properties of the mRNA vaccines (i.e size and electrostatic charge, which hinder RNA's ability to generate an immune response) with using particular type of NPs. Even though there are legitimate concerns about the price and safety of NPs in medicine, nanotechnology has been used valuably to increase vaccine efficiency against COVID-19 (Ruiz-Hitzky et al., 2020; Tharayil et al., 2021; Yang, 2021).

Nanotechnology provides benefits and simplifies the overall healing of pharmacological drug properties through nanosystems are used for drug encapsulation (i.e., liposomes, metallic/polymeric-NPs, and micelles). Antiviral agents for NPs can target binding. The major reason why inorganic NPs need modification is concerns about toxicity. Due to the close morphological and physicochemical properties of SARS-CoV-2 and synthetic NPs, it allows NPs to be an effective intervention method (Wang J. et al., 2020; Xie et al., 2020; Behbudi, 2021; Doagooyan, 2021; El-Megharbel et al., 2021).

6.1 Robotics applications in COVID-19

Robots can take on human-like activities and can be programmed profitably to replace some human interactions (Javaid et al., 2020; Hussain et al., 2021). They play a variety of roles in the medical field to perform specialized human treatment and surgery. Robotic technology helps to make up for the lack of doctors, it can also assist a surgeon during a complex operation and perform tasks that are risky for humans as in remote locations (Javaid et al., 2020). Certain robots are useful in managing COVID-19 patient cure in analysis (Javaid et al., 2020; Zhao et al., 2021b; Doğuç, 2021; Sarker et al., 2021; Shorten et al., 2021). For example, this technology is useful for overcoming various difficulties during the quarantine situation. Robots help maintain social distancing and monitor large populations. They are also used for packaging necessary medical equipment. Moreover, robots help incapacitated people and play an important role in their recovery process (Javaid et al., 2020; Firouzi et al., 2021; Khamis et al., 2021; Mbunge et al., 2021; Sarker et al., 2021; Shorten et al., 2021).

6.2 Tissue engineering and COVID-19

Tissue engineering (TE) is a field with a unique set of tools and technologies to develop diagnostics and treatments during the COVID-19 (Aydin et al., 2021; Shafiee et al., 2021). Tissue engineering is pioneered by the pandemic and can play an important role in preventive strategies and identifies methods that can be applied to the current COVID-19 crisis as well as future viral outbreaks (Harikrishnan and Krishnan, 2020; Tatara, 2020; Softa et al., 2021). TE covers the behavior and growth factors phenomenon of TE *in vitro* (Harikrishnan and Krishnan, 2020; Tatara, 2020; Aydin et al., 2021; Softa et al., 2021). This field of TE is able to support the treatment of COVID-19 patients and help combat current crises. Current TE studies report the greatest challenges faced during the COVID-19 situation, important developments in TE, and in medicine are listed in chronological order, the positive effects of TE during the COVID-19 crisis. The primary importance of this branch of science is to offer biological alternatives that can fully or partially fulfill the functions of damaged, dysfunctional organs or tissues in humans. It is particularly useful for the supply of convalescent plasma to patients during COVID-19. A donor is chosen based on a strictly confirmed case of COVID-19 transmission. The donor must confirm a negative molecular examination that does not show any symptoms (Harikrishnan and Krishnan, 2020; de Melo et al., 2021; Shafiee et al., 2021). With the contributions of talented scientists, TE's future potential for the COVID-19 will be the realization of successful initiatives. More work needs to be done in the areas of healthcare and different methods to develop diagnostic products such as PCR kits and to implement TE in the COVID-19 (World Health Organization, 2020; Wang, 2021; Yunus et al., 2021; Zhang T. et al., 2021).

The application of TE provides extended support for the development of diagnostic products that help improve clinical processes (Tatara, 2020; Shafiee et al., 2021). Researchers use the

TE animal model to describe it. In this ongoing COVID-19, TE has an important role in the application of the Bioscaffold matrix, the treatment of COVID-19 infection, the healthy functioning of the body and the examination of healing mechanisms. In the future, TE will play an important role in saving the life of a COVID-19 patient (Malavazos et al., 2020; Tatara, 2020; Bailey et al., 2021; Dorward et al., 2021; Kerch, 2021).

6.3 Antimicrobial nanotechnology for COVID-19

Antimicrobial studies have several roles during the pandemic. Primary role, agents such as azithromycin, lopinavir, and ritonavir as well as antivirals and remdesivir are being investigated in clinical trials as potential treatments for SARS-CoV-2. However, the trials did not clearly demonstrates improved clinical results compared to the standard treatment methods (Anvar et al., 2021; Erkoc and Ulucan-Karnak, 2021; Khorsandi et al., 2021). Second, antimicrobials are directly related to COVID-19, they are widely prescribed for the management possible or approved bacterial infections that can happen during long-term acceptance to intensive care. Available proof indicates that bacterial and/or fungal co-infection is low in patients and diseases with COVID-19. This is due to the lack of rapid diagnosis resulting in an increase in unnecessary antimicrobial use, which resulted the future risk of antimicrobial resistance (AMR) through the selection of *Enterobacteriaceae* that produces carbapenemase (Kaur et al., 2020; Kchaou et al., 2020). The potential avoidance of healthcare by patients due to self-isolation healthcare due to service changes limits the requisition but also limits the access to essential anti-microbials. Increased use of telemedicine, along with continued public interest discourses on the role of antibiotics in viral infections may reduce the use of antimicrobials and AMR (Karaman et al., 2021). In addition to this, hand hygiene can prevent the transmission of AMR as well as reduce the diseases that can lead to antibiotic use. This will likely have a significant impact on countries with limited resources and large numbers of endemic infectious diseases. After the initial global increase in COVID-19 cases, the importance of such measures is once again increasing until vaccine implementations take place. Delays in elective surgery and cancer treatment will increase the pressure, potentially resulting in increased healthcare needs and increased use of antimicrobials (Almeida et al., 2020; Johnson, 2021; Knight et al., 2021; Lai et al., 2021; Schouten et al., 2021; Khan et al., 2022).

7 Conclusion

Nanotechnology offers new solutions during fight against COVID-19 by providing better improved forms of treatment, prevention, and diagnosis. Advances in bio/nanotechnology and advanced nano/manufacturing, combined with open reporting and data sharing, lay the foundation for the rapid development of innovative technologies that will have an impact during the COVID-19 pandemic. Thanks to the increasing relationship between the COVID-19 pandemic and biotechnological methods, we should say that these biotechnological methods are used in the majority of vaccines developed for COVID-19. In addition, there are different approaches in the diagnosis and treatment of SARS-CoV-2. There still needs to be some development work done—for example, a system that concentrates aeroceles in the air and releases RNA from viruses. It is thought that the discovery of new methods with the development of technology and the use of these discovered methods for the COVID-19 will have an important share in the fight against this pandemic.

Author contributions

Conceptualization, SA, KA-C, and CU; data curation, SA, KA-C, and FC; writing—original draft, SA and KA-C; writing—review and editing, SA, KA-C, FC, and CU; visualization, SA, KA-C, and FC; supervision, All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Potential of siRNA in COVID-19 therapy: Emphasis on *in silico* design and nanoparticles based delivery

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Small interfering RNA (siRNA)-mediated mRNA degradation approach have imparted its eminence against several difficult-to-treat genetic disorders and other allied diseases. Viral outbreaks and resulting pandemics have repeatedly threatened public health and questioned human preparedness at the forefront of drug design and biomedical readiness. During the recent pandemic caused by the SARS-CoV-2, mRNA-based vaccination strategies have paved the way for a new era of RNA therapeutics. RNA Interference (RNAi) based approach using small interfering RNA may complement clinical management of the COVID-19. RNA Interference approach will primarily work by restricting the synthesis of the proteins required for viral replication, thereby hampering viral cellular entry and trafficking by targeting host as well as protein factors. Despite promising benefits, the stability of small interfering RNA in the physiological environment is of grave concern as well as site-directed targeted delivery and evasion of the immune system require immediate attention. In this regard, nanotechnology offers viable solutions for these challenges. The review highlights the potential of small interfering RNAs targeted toward specific regions of the viral genome and the features of nanoformulations necessary for the entrapment and delivery of small interfering RNAs. *In silico* design of small interfering RNA for different variants of SARS-CoV-2 has been discussed. Various nanoparticles as promising carriers of small interfering RNAs along with their salient properties, including surface functionalization, are summarized. This review will help tackle the real-world challenges encountered by the *in vivo* delivery of small

Abbreviations: siRNA, Small interfering RNA; RNAi, RNA interference; dsRNA, Double-stranded RNAs; RISC, RNA-induced silencing complex; Ago2, Argonaute-2; TRBP, Transactivation response RNA-binding protein; SARS-COV 2, Severe acute respiratory syndrome coronavirus 2; LNP, Lipid nanoparticle; ORF, Open reading frame; hpTCs, Human primary airway-tracheal cells; RdRp, RNA-dependent RNA polymerase; UTR, Untranslated region; LNA, Locked nucleic acid; PLP, Papain-like protease; TNF-α, Tumor necrosis factor-α; IL-6s, Interleukin-6; BLAST, Basic Local Alignment Search Tool; PEG, Poly ethylene glycol; DLin-MC3-DMA, (6Z,9Z,28Z,31Z)-heptatriacont-6,9,28,31-tetraene-19-yl 4-(dimethylamino)butanoate; ALC-0315, [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate); SM-102, (1-Octylnonyl 8-[(2-hydroxyethyl)[6-oxo-6(undecyloxy)hexyl]amino]-octanoate); DOPE, Dioleoylphosphatidylethanolamine; DSPC, Distearoylphosphatidylcholine; PLA, Poly(lactic acid); PGA, Poly(glycolic acid); PLGA, Poly(lactic-co-glycolide); FDA, Food and Drug Administration; PEI, Polyethyleneimine; ApoE, Apolipoprotein E; DODAP, 1,2-dioleoyl-3-dimethylammonium-propane; RBD, Receptor binding domain; SELEX, Systemic Evolution of Ligands by EXponential enrichment; DMKE, O,O′-dimyristyl-N-lysyl glutamate; SORT, Selective organ targeting; DOTAP, Dioleoyl-3-trimethylammonium propane; hATTR, Hereditary transthyretin amyloidosis.

interfering RNAs, ensuring a safe, stable, and readily available drug candidate for efficient management of SARS-CoV-2 in the future.

KEYWORDS

siRNA, lipid nanoparticles, surface modification, ligands, COVID-19

Introduction

RNAi therapeutics have had a promising impact in reducing the expression of disease-associated genes ever since their discovery in 1990s (Kalita et al., 2022). The field received its major breakthrough in 2018 with the approval of the first siRNA-based drug 'Patisiran' (Onpattro®) for the treatment of transthyretin-mediated amyloidosis (Zhang et al., 2022a). Since then, over 30 drug candidates have been in the clinical trial pipeline as next-generation medicines to develop medications for difficult-to-treat ('undruggable') genetic disorders and ever-evolving SARS-CoV-2-like viral pandemics (Bunea et al., 2020). In this treatment approach, the double-stranded RNAs (dsRNA) designed explicitly against specific disease-causing mRNA sequences are loaded onto a gene regulatory complex, i.e., RNA-induced silencing complex (RISC), consisting of DICER, Argonaute-2 (Ago2), and transactivation response RNA-binding protein (TRBP) proteins (Jayaraman et al., 2012). The dsRNA is cleaved by the RISC complex producing two different strands, out of which the passenger strand is lost, with the guide strand getting paired with the target mRNA meant to be cleaved (Dobrowolski et al., 2021; Ly et al., 2022). Finally, Ago2, the catalytic precursor of the process, cleaves the bound mRNA (Han et al., 2020).

The promising therapeutic benefits of siRNAs are overshadowed by the difficulty in attaining optimal biodistribution and pharmacokinetics of the RNAi therapeutic agents. Various intracellular hurdles, such as non-targeted accumulation in the liver/spleen, impaired long-term protein expression, immunological response, endosomal escape, and post-administration reactions, pose a significant challenge to siRNA therapy (Kalita et al., 2022). To effectively counter such challenges, proper computational approach-based siRNA designing is deemed critical (Idris et al., 2021). Further, the stability and early *in vivo* elimination issues are resolved by loading siRNA molecules onto a suitable nanocarrier targeted against specific tissues or cells (Evers et al., 2022). Nanocarriers tend to improve the overall potency of naked siRNA molecules, reducing the nuclease digestion, off-target binding and unwarranted immune reactions (Gupta et al., 2019). In addition, the surface functionalization of nanoparticles using suitable ligands ensures a site-specific delivery (Khanali et al., 2021).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pathogenic and transmissible form of coronavirus which emerged in late 2019, creating havoc in the World and posing a major public threat to health and safety (Hu et al., 2021). Novel mRNA-based vaccines such as that from Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273), have been quickly developed to restrict the growth of the virus (Corbett et al., 2020; Walsh et al., 2020; Baden et al., 2021). Likewise, to aid the scientific community in developing safe treatment strategies against the virus, drug candidates can also be developed using the concept of siRNA-mediated therapeutics that will utilize the endogenous RNAi pathway. The present review, on one hand, discusses *in silico* strategies for siRNA design against various functional genomic regions of the SARS-CoV-2. On the other hand, select carrier molecules, such as lipid-based and polymeric nanoparticles for siRNA entrapment and delivery are highlighted with their preparation methods since, without an appropriate carrier, RNAi therapeutics display sub-optimal pharmacological activities. The review also briefly explains the targeting of siRNA-loaded lipid nanoparticles (LNPs) and the release of siRNA *in vivo* for desired inhibition. Two comprehensive tables are penned down detailing the siRNA sequences specifically evaluated against SARS-CoV-2 viral genome segments, along with approved siRNA drugs, and ongoing clinical trials. The siRNA completely recognizes and base pairs with the mRNA of interest, followed by its degradation. In contrast, small molecule and antibody-based drugs recognize only a specific protein conformation, making the siRNA robust to address any disease-associated genes. Moreover, siRNA needs to be administered less frequently, in contrast to antibody-based drugs requiring frequent administration. With such advantages come quicker research and developmental span, together with a broader economic and therapeutic perspective (Hu et al., 2020). The review equivocally concludes the significance of siRNA-based nanoparticle formulation as a better alternative against SARS-CoV-2.

Design of small interfering RNA for different variants of SARS-CoV-2

The single-stranded positive RNA (+ssRNA) genome of the COVID-19 virus, a member of the β-coronavirus family, encodes about 29 proteins out of which four are structural proteins, the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (Muhseen et al., 2020; Thi Nhu Thao et al., 2020; V'kovski et al., 2021). Additionally, the genome has at least five open reading frames (ORFs) with the first ORF (ORF1a/b) occupying 70% of the entire genome. Considering the overall structure of the viral genome to be 5′-UTR-ORF1ab-S-E-M-N-3′-UTR-poly adenine-tail, ORFs constitute nearly two-thirds and are necessary during viral replication. There could broadly be two categories of RNAi targets for coronavirus. The first one involves viral proteins required for growth and replication, whereas the other is related to viral cellular entry and trafficking (Uludağ et al., 2020).

Since ORFs modulate the infectivity of the virus, RNAi-based strategies have been employed against the ORFs. An *in vitro* study designed siRNAs targeted against the ORF1a/b region of the viral genome (coding for a non-structural protein) which reduced the viral burden by 99% and 97% in Vero E6 and Huh-7 hepatoma cells, respectively (Friedrich et al., 2022). A bioinformatics screening study of siRNA libraries on the basis of melting temperature (T_m), GC content, heat capacity (C_p), and free energy of hybridization has identified potential therapeutic agents against SARS-CoV-2 including pre-miRNA hairpins and siRNAs (Hasan et al., 2021). The siRNAs were found to target multiple SARS-CoV-2 variants, *viz.* Wuhan-Hu-1 (MN908947.3), alpha (MW686007.1), beta

(MW880890), gamma (LR963075.1), delta (MW994451), and omicron (OV112121) (Friedrich et al., 2022). However, this study did not consider any *in vivo* delivery approach. Another *in vitro* study using HEK-293 and Vero E6 cells reported the design of siRNA sequences to target the ORF1 considering the alpha (B.1.1.7 and Q. x), beta (B.1.351, B.1.351.2 and B.1.351.3), gamma (P.1 and P.1. x), delta (B.1.617.2 and AY. x), lambda (C.37 and C.37.1), and mu (B.1.621 and B.1.621.1) variants of the virus. At a concentration of 50 nM, one designed sequence "5′-GGUACUUGGUAGUUUAGC UTT-3'" inhibited the viral replication by 92.8% (Ambike et al., 2022). However, the study did not consider any *in vivo* delivery approach.

Apart from ORFs, the four structural proteins (S, E, M, and N) have also been identified as RNAi targets (Sajid et al., 2021). Focusing on the spike (S) protein, the receptor-binding domain in the S1 segment binds to the ACE2 receptor of the plasma membrane to enter the host cells and illicit the host immune cell response. Recent siRNA studies on HEK-293 cells and the human primary airway-tracheal cells (hpTCs) have been depicted to reduce the protein expression of spike protein in a dose-dependent manner (Gallicano et al., 2022). The authors imply the use of cholesterol moiety to modify the siRNA to reduce the use of toxic siRNA transfection agents. Lipid-modified siRNAs can also exert equally robust inhibition (Gallicano et al., 2022). Several bioinformatics-based studies have also been reported in regard to the spike protein (Chen et al., 2020; Niktab et al., 2021; Panda et al., 2021; Ayyagari, 2022). However, the spike protein is prone to mutations, as observed in SARS-CoV-2 variants (i.e., alpha (B.1.1.7), beta (B.1.351), gamma (P.1), and delta (B.1.617.2)). Similarly, ACE-2, the receptor for the S-protein also acts as an important siRNA target (Xiao et al., 2020). An *in vitro* approach designed effective siRNAs targeted against the ACE-2 mRNA, reduced the mRNA expression by 90% in 6 days, with 92% viral burden reduction in Vero E6 and Huh-7 hepatoma cells (Friedrich et al., 2022). Whether targeting viral or host targets are more effective (preferable) is an open question.

The viral M protein is responsible for maintaining the structural integrity of the viral membrane and helps bind to nucleocapsids. With the N-terminal ectodomain and C-terminal endodomain, the viral M-protein may act as a suitable siRNA target (Ullah et al., 2020). Likewise, the E protein helps in viral assembly and release, and may also act as siRNA target. As mutations can alter the siRNA sequence thereby reduce its efficiency, hence targeting a strongly conserved region such as the 5′-UTR (untranslated region) is always preferred (Zhang et al., 2022a).

Several *in silico* studies have pioneered the field of siRNA prediction, synthesis and design for SARS-CoV-2. An *in silico* analysis study against the leader sequence of the virus depicted the highest binding score as indicated by the HNADOCK server (Pandey and Verma, 2021). Another *in silico* study designed siRNAs against the viral S-protein, ORF1ab, ORF3a, E-protein, and M-protein (Niktab et al., 2021). Moreover, another group designed siRNA sequences against the RNA-dependent RNA polymerase (RdRp) gene of SARS-CoV-2, and checked their binding scores with the RdRp gene segment by docking and molecular dynamics simulation (Shawan et al., 2021). One of the preliminary *in silico* approaches by Chen et al. predicted 9 siRNA sequences directed against the ORF1a/b, S, ORF3a, M, and the N-protein regions of the viral genome (Chen et al., 2020). The authors also incorporated single point mutations across different variant strains of the virus. In a separate *in silico* study, 139 SARS-CoV-2 sub-strains were considered and found a total of 34 conserved regions (15 in nucleocapsid and 19 in surface glycoprotein) (Chowdhury et al., 2021). The authors have then developed 78 siRNA sequences targeting the surface glycoprotein and nucleocapsid (N) phosphoprotein based on the U, A, and R rules. The authors also modelled the Ago2 and performed molecular docking of siRNAs with Ago2 to find out the best siRNA sequences (Chowdhury et al., 2021).

Several *in vitro* and *in vivo* studies have also focused on the screening, modification and delivery of targeted siRNAs for obstructing viral growth. A study combining the computational screening with *in vivo* targeting approach found 13 suitable siRNAs against the viral RdRp and the N protein (Khaitov et al., 2021). The siRNAs, however, were modified with locked nucleic acids (LNAs) for imparting stability and were delivered through a non-toxic peptide dendrimer KK-46 carrier into the destined cells in culture or in Syrian hamsters by inhalation (Khaitov et al., 2021). A daily concentration of ~3.5 mg/kg of siR-KK-46 reduced lung inflammation as indicated by histopathological microscopic observation on day 6 as compared to the non-treated animals (Khaitov et al., 2021). However, the study did not verify the siRNAs against other prevalent SARS-CoV-2 strains. Another study designed 8 siRNAs targeting the 5′-UTR region of the virus with one molecule inhibiting the replication of SARS-CoV-1 and alpha variant of SARS-CoV-2 at 10 nM of concentration as indicated by *in vitro* studies in Vero E6 cells (Tolksdorf et al., 2021). However, *in vivo* delivery studies were not performed. In one major study (Idris et al., 2021), siRNAs were designed targeting the conserved regions of the virus, i.e., RdRp, helicase, and the 5-UTR. Three sequences were found to reduce viral growth by 90% in Vero E6 cells. The siRNAs were chemically modified with 2′-O-methyl and phosphorothioate to impart stability against the nucleases (Idris et al., 2021). Furthermore, the modified siRNAs were formulated with a delivery vehicle based on LNPs for *in vivo* studies. Intravenous retro-orbital administration of 100 µL of the siRNA-LNP formulation at a concentration of 1 mg/kg in K18-hACE2 mice, restored mice weight and modulated the immune gene expression (Figure 1) (Idris et al., 2021). The formulation targeted against the helicase and UTR3 also improved clinical score at 6 days post-infection, with a reduction in the amount of infectious virus particles as titrated by immunoplaque assay (Idris et al., 2021). An inhalable formulation of siRNA at a concentration of ≤30 mg/mL decreased the viral burden by 96.2% in K18-hACE2-transgenic mice, along with a reduction in associated damage (Chang et al., 2022). The siRNA sequences were designed to target specific regions the leader sequence, RdRp, helicase, S, E, N regions, papain-like protease (PLP), and 3C-like protease of SARS-CoV-2 strains, including Alpha, Delta, Gamma, and Epsilon strains (Chang et al., 2022). After the removal of off-target sequences, a total of 11 sequences was able to reduce the viral load by 99% in Vero E6 cells, even at 10 nM of concentration. The modified siRNA was well tolerated and was not found to induce immune stimulation across the range of 20–75 mg/kg, as verified by mRNA expression of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interferon- γ (IFN-γ) (Chang et al., 2022).

To cater the needs of the growing bioinformatics research, several databases encompassing the list of sequences, thermodynamic features, GC percentage, target genomic data, possible off-target effects, and applicability against multiple strains have emerged (Dar et al., 2016a; Medeiros et al., 2021). These databases also catalog the

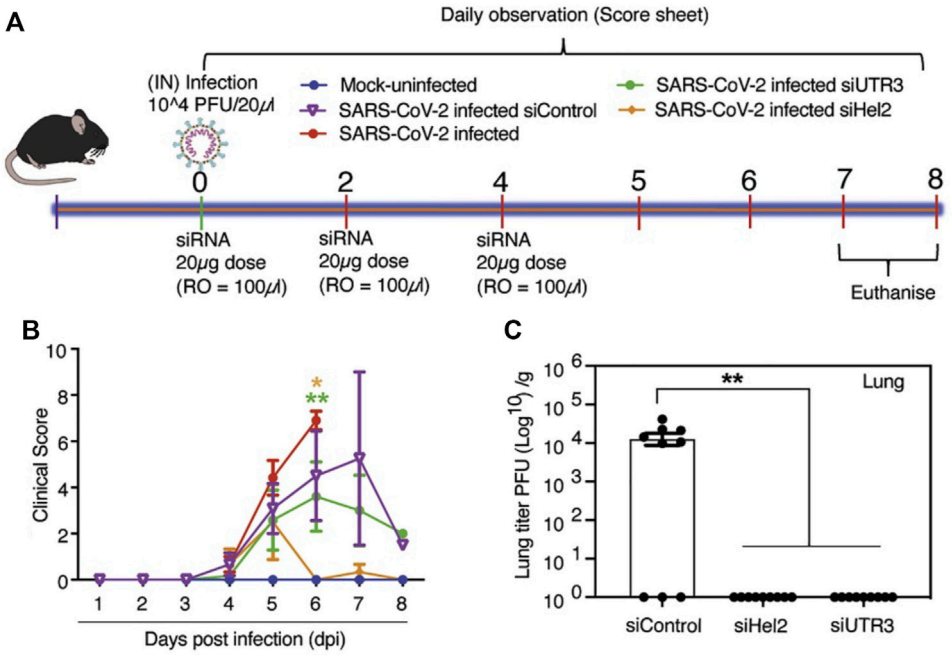

FIGURE 1
(A) Experimental timeline of *in vivo* study in K18-hACE2 mice employing LNP to deliver siRNA. **(B)** Intravenous administered LNP-siRNA formulation improved the clinical score at 6 days post-infection. **(C)** The amount of infectious virus particles in lung tissues at 6 days post-infection as titrated by immunoplaque assays on Vero E6 cells. siRNAs targeted to helicase-2 and UTR3 exhibited decreased or no residual viral particles. Adapted with permissions from Ref. (Idris et al., 2021).

toxicity assessment information by checking the *in silico* off-target binding against the human genome, stability, probable structure, chemical modification, and experimental verification information (Dar et al., 2016a; Medeiros et al., 2021). Table 1 summarizes the aforementioned siRNA guide strand sequences along with their target sites, key results and drawbacks of the studies.

The siRNAs are designed usually against the conserved region of the viral genome targeting the mRNA sequence responsible for the formation of the structural proteins (S, M, E, N), which help the viral particle to assemble and impart infectivity. To design well-targeted and precise siRNAs, computational approaches are followed with a preliminary retrieval of the genomes of the SARS-CoV-2 virus and variants. Conserved genomic regions across different variants are obtained by multiple sequence alignment with Clustal omega (Sievers and Higgins, 2018). Typically, these conserved regions across the different variants are regarded as the potential siRNA target sites. Next, with the help of web servers, siRNAs are designed considering Ui-Tei (Ui-Tei, 2004), Amarzguioui (Amarzguioui and Prydz, 2004), and Reynolds (Reynolds et al., 2004) rules. Many *in silico* siRNA prediction studies use multiple web servers to predict siRNAs targeted towards the same mRNA sequence and finally consider the common predicted siRNAs (Ayyagari, 2022). This approach ensures stringent shortlisting and robust applicability (Ayyagari, 2022). A few of the web servers include OligoWalk (Mathews and Sioud, 2010), i-Score Designer (Ichihara et al., 2007), siDirect v2.0 (Naito et al., 2009), and RNAxs (Tafer et al., 2008). To counter verify the thermodynamic suitability and readiness of the siRNAs, additional parameters are evaluated. The free energy of folding of the siRNA guide strand, along with the secondary structure prediction is performed using MaxExpect (Lu et al., 2009), DuplexFold, AccessFold (DiChiacchio et al., 2016), and ViennaRNA (Gruber et al., 2015) web-servers to rule out any RNA-RNA self-hybridization. Moreover, the efficiency of inhibition by the siRNA is predicted using SMEpred, siRNAPred, and VIRsiRNApred (Qureshi et al., 2013; Dar et al., 2016b). Further, heat capacity (C_P)/melting temperature (T_m) and GC content of the siRNA are predicted using DINA melt server (Markham and Zuker, 2005) and OligoCalc (Kibbe, 2007), respectively. Moreover, BLAST® (Basic Local Alignment Search Tool) search against human genome is performed to identify off-target matches of the siRNA. Finally, 3D structure of the siRNA is predicted and computationally docked with Ago2 (PDB: 4OLA) followed by molecular dynamics simulation. Figure 2 summarizes the overall computational strategy to predict siRNA against target segments of SARS-CoV-2.

Nanoparticles (NPs) used for the siRNA therapy of COVID-19

The administration of naked siRNA for *in vivo* application is challenging due to various biological barriers such as degradation by RNAases, instability of the molecule and the immune response that could neutralize the siRNA and can cause other adverse effects (Kalita et al., 2022). The carriers provide protection to the siRNA against biological factors and facilitate targeted delivery (Zhang et al., 2022a). For the treatment of COVID-19, the vaccines, Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) use lipid nanoparticles as carrier molecules. There are a

TABLE 1 siRNA guide strand sequences reported in literature along with their target site, and drawback of the study.

siRNA guide strand sequence	Target site of the virus	Key results	Drawbacks	Ref
5′-GCGAAAUACCAGUGGCUUA-3′	ORF1	Reduction of the viral burden by 99% and 97% in Vero E6 and Huh-7 hepatoma cells	Lack of *in vivo* approach	Friedrich et al. (2022)
5′-UCAAUAGUCUGAACAACUGGU-3′ 5′-UACCUUUUUAGCUUCUUCCAC-3′ 5′-UGUUUAGCAAGAUUGUGUCCG-3′	ORF1a/b	siRNA selected based on their efficient binding score with target sequence	Only *in silico* prediction-based study	Hasan et al. (2021)
5′-GGUACUUGGUAGUUUAGC UTT-3′	ORF1	92.8% SARS-CoV-2 viral replication inhibition at 50 nM	Lack of *in vivo* approach	Ambike et al. (2022)
5′-UUCGUUUAGAGAACAGAU CTT-3′	5′UTR	Viral replication inhibition at 10 nM concentration of siRNA in studies in Vero E6 cells	Lack of *in vivo* delivery approach	Tolksdorf et al. (2021)
5′-GGAAGGAAGUUCUGUUGA ATT-3′	RdRp, and N-protein	siRNA modified with locked nucleic acid and KK-46 dendrimer (3.453 mg/kg concentration) to enhance uptake by inhalation in Syrian hamsters	Effectivity was not checked for other variants	Khaitov et al. (2021)
5′-GUUUAGAGAACAGAUCUA CAA-3′	Leader sequence	siRNA sequence has the highest docking score with the leader sequence and no off-target binding	No *in vitro* or *in vivo* studies performed and not checked against variants	Pandey and Verma, (2021)
5′-GGACAAGUUUAACCACGAA-3′	ACE2	Reduced mRNA expression by 90% in 6 days, with 92% viral burden reduction in Vero E6 and Huh-7 hepatoma cells	Lack of *in vivo* approach	Friedrich et al. (2022)
5′-GTACTTTCTTTTGAACTTCTA CA-3′	S-protein	siRNA sequences selected based on their efficient binding score with target sequences	No *in vivo* or *in vitro* analysis performed or variants considered	Niktab et al. (2021)
5′-CAACAAAGATAGCACTTAA-3′	ORF1ab			
5′-TCATACCACTTATGTACAA-3′	ORF1ab			
5′-CCAAAATCATAACCCTCAAA-3′	ORF3a			
5′-AAACCTTCTTTTTACGTTTA-3′	E-protein			
5′-CGAACGCTTTCTTATTACAA-3′	M-protein			
5′-UAGUACUACAGAUAGAGA CAC-3′	RdRp	Key siRNA was selected based on the efficient binding score with Ago2 by molecular dynamics simulation	No *in vivo* or *in vitro* experiments performed	Shawan et al. (2021)
5′-UCCUUCUUUAGAAACUAU ACA-3′	ORF1a/b	siRNA selected based on the binding score	Theoretical *in silico* work	Chen et al. (2020)
5′-UGGUUUCACUACUUUCUG UUU-3′	ORF1a/b			
5′-CUUGAAGCCCCUUUUCUCUAU CUUU-3′	ORF3a			
5′-UUAAAAUAUAAUGAAAAU GGA-3′	S-protein			
5′-CAACUAUAAAUUAAACAC AGA-3′	M-protein			
5′-UUGAAUACACCAAAAGAUCAC AUU-3′	N-protein			
5′-UUUGUAUGCGUCAAUAUG CUU-3′	N-protein Surface glycoprotein	siRNA selected based on the molecular docking score between surface proteins and siRNA sequence	*In silico* work without consideration of *in vitro* or *in vivo* work	Chowdhury et al. (2021)
5′-UAAUUUGACUCCUUUGAG CAC-3′				
5′-GGUGGUGUCAGUGUUAUAA-3′	S-gene	Four sRNA sequences predicted for the spike protein of the virus	*In silico* prediction, and not much detailed about different strains	Panda et al. (2021)
5′-GCAAAUGGCUUAUAGGUU UAA-3′				
5′-GAGUUACACAGAAUGUUC UCU-3′				
5′-CACAGAAUGUUCUCUAUG AGA-3′				

(Continued on following page)

TABLE 1 (*Continued*) siRNA guide strand sequences reported in literature along with their target site, and drawback of the study.

siRNA guide strand sequence	Target site of the virus	Key results	Drawbacks	Ref
5′-UGUAAUAAGAAAGCGUUC GUG-3′	M-gene	Six siRNAs found for all three S, M, and N-gene of the virus on basis of *in silico* prediction score	Neither *in vitro* or *in vivo* approach, nor any other variants data mentioned	Ayyagari, (2022)
5′-UAAUAAGAAAGCGUUCGU GAU-3′	M-gene			
5′-UGAAAUUUGGAUCUUUGU CAU-3′	N-gene			
5′-UUUCUUAGUGACAGUUUG GCC-3′	N-gene			
5′-UAGAAGUUUGAUAGAUUC CUU-3′	S-gene			
5′-UUUUUGUCUUGUUCAACA GCU-3′	S-gene			
5′-UAUGGGUUGGGAUUAUCCUAA AUGT-3′	RdRp	siRNA-HFDM LNP formulation at a concentration of 1 mg/kg in 100 µL administered retro-orbitally in mice reduced viral burden	Consideration of different variant data is absent	Idris et al. (2021)
5′-UGUUGAUUCAUCACAGGGCUC AGAA-3′	Helicase			
5′-GUCCCUGGUUUCAACGAGAAA ACAC-3′	UTR1			
5′-AUACCUUCCCAGGUAACAAAC CAAC-3′	UTR3			
5′-CUGUCAAACCCGGUAAUUU-3′	RdRp	Inhalable formulation of siRNA reduced the viral load and damage by 96.2% in mice	Inhalation route was considered, use of a targeted delivery system is not mentioned	Chang et al. (2022)
5′-CAGCAUUAAAUCACACUAA-3′	PLP			
5′-GCCACUAGUCUCUAGUCAA-3′	S-protein			
5′-CGCACAUUGCUAACUAAGA-3′	Helicase			
5′-UUUGUACUGGUCAAUAUG CUU-3′	S-protein	Aptamer-siRNA-LNP conjugate reduced the viral load by 50% *in vitro* and in a patient	Liposomes were used for delivery	Saify Nabiabad et al. (2022)

In silico design of siRNA.

number of suitable carrier configurations for the siRNA-based therapy as shown in Figure 3. Some of the potential nanocarriers are briefly discussed in this section.

Lipid nanoparticles

The lipid composition of the cellular membrane bilayer is the basis for designing and formulating the LNPs. The favorable interactions of the LNPs with the cell membrane determines the internalization, release, and stability of the payload (Bunea et al., 2020; Lee et al., 2022). Currently, LNPs are a popular strategy being explored for various diseases and applications (Chernikov et al., 2019), mostly attributable to the success of the mRNA-based vaccines against COVID-19. The LNPs are mainly composed of four components: Cationic or ionizable lipid (IL), cholesterol, helper lipid, and PEGylated lipid (Ly et al., 2022). Figure 4 represents the key constituents of LNPs. The cationic or ILs (also known as pH-sensitive lipids) interact with RNA molecules through electrostatic interaction between amine and phosphate groups. The interactions mediate the cellular internalization of nanoparticles along with siRNA and their release in the cytoplasm (Jayaraman et al., 2012; Lee et al., 2022). DLin-MC3-DMA [(6Z,9Z,28Z,31Z)-heptatriacont-6,9,28,31-tetraene-19-yl 4-(dimethylamino) butanoate], ALC-0315 {[(4-hydroxybutyl) azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate)}, and SM-102 {1-Octylnonyl 8-[(2-hydroxyethyl) (6-oxo-6 (undecyloxy)hexyl) amino]-octanoate} are clinically approved lipids, among which ALC-0315 and SM-102 have been applied for mRNA delivery, and DLin-MC3-DMA (MC3) has been used for siRNA delivery for treating transthyretin amyloidosis (Suzuki and Ishihara, 2021; Urits et al., 2021; Saadati et al., 2022). The helper lipids are generally phospholipids such as DOPE (dioleoylphosphatidylethanolamine) and DSPC (distearoylphosphatidylcholine) and are used for the stability of LNPs and for aiding the endosomal release (Hou et al., 2021). DSPC has been used in the approved vaccines, improving structural stability through the formation of the lamellar phase while DOPE is proven for endosomal disability and release (Koltover et al., 1998). Cholesterols are responsible for structural integrity and promotes membrane fusion (Cheng and Lee, 2016). The PEG moieties enhance the colloidal stability and prevent the aggregation of serum protein on the LNPs surface and immune response (Suk et al., 2016; Sebastiani et al., 2021).

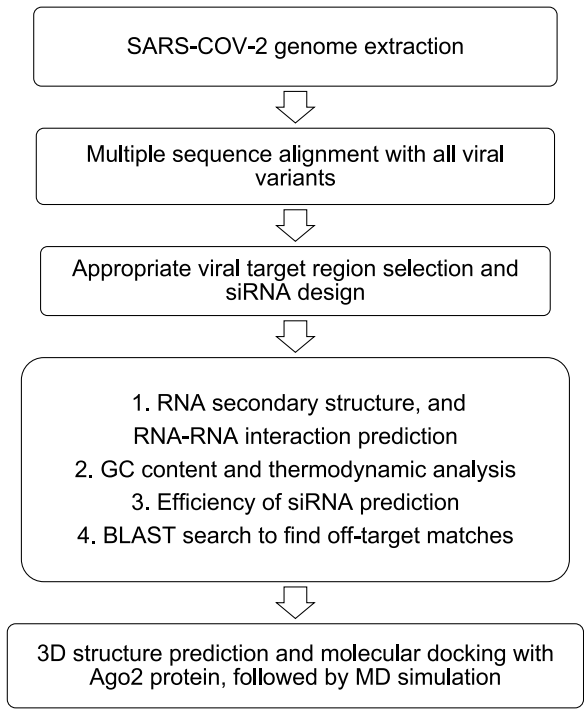

FIGURE 2
Computational pipeline to predict siRNA against target segments of SARS-CoV-2. Adapted with modifications from Ref. (Chowdhury et al., 2021).

Along with the conventional composition, Bogaert *et al.* have attempted the repurposing of cationic amphiphilic drugs (tricyclic antidepressants and antihistamines) for formation of cationic lipid vesicles for mRNA delivery. These drug molecules, due to their amphiphilic properties, accumulate in acidic lysosomes in their active form. The formed complex can be used to co-deliver mRNA within cationic amphiphilic drugs-assisted LNPs for various applications (Bogaert et al., 2022). The approved LNP-based therapeutic formulations such as Patisiran (Alnylam), Elasomeran (Moderna) use similar ratios (50:10:38.5:1.5) of ILs, helper lipid (DSPC), cholesterol and polyethylene glycol lipids (Suzuki and Ishihara, 2021; Ferraresso et al., 2022). The said molar ratio has been reported by many studies as optimum and highly potent (Carrasco et al., 2021; Suzuki et al., 2022). Pfizer-BioNTech mRNA vaccine Tozinameran is based on a lipid molar ratio of 46.3:9.4:42.7:1.6 (ALC-0315:DSPC:Cholesterol:ALC-0159) (Schoenmaker et al., 2021).

Properties of LNPs. The selection of ILs or pH-sensitive lipids depends on the acid-dissociation constant (pKa) value. These pH-sensitive lipids possess deprotonated tertiary amine head groups at the physiological pH, but acquire positive charges at pH below pKa. Thus, the ILs interact with the negatively charged siRNA molecules through electrostatic interactions enabling neutralization. The neutral surface charge under the physiological conditions of blood and serum eases the LNP internalization through the plasma membrane. The protonation of the head groups in the acidic condition in the cytoplasm helps destabilize the LNPs structure to release siRNA molecules (Zhi et al., 2013; Albertsen et al., 2022; Syama et al., 2022). The pKa value of the head group of ILs determines the surface charge, which ultimately affects biodistribution, cellular internalization, and endosomal release. Studies have reported that lipids with pKa values between 6 and 6.6 showed well *in vivo* activity (Rajappan et al., 2020). Carrasco *et al.* studied the pKa values of some commercially available lipids and stated that the lipids with pKa values between 6 and 7 are optimum for RNA-based therapeutics, considering the endosomal release at acidic conditions. The variations in the zeta potential of nanoparticles derived from commercial lipids and the pKa values of the lipids are as shown in Figure 5 (Carrasco et al., 2021). The ionizable lipid DLin-MC3-DMA with an apparent pKa 6.44 used in Onpattro® was identified from a library of 56 ILs consisting of a dilinoleyl-based hydrophobic tail with varying headgroups (Jayaraman et al., 2012).

The tail length of the lipids determines the fluidity in the bilayer *via* the carbon length and the structure of the aliphatic chain (Zhi et al., 2010). The linker between the head group and tail affects the stability, cytotoxicity, and other aspects. The internalization and the release of the nucleic acid molecules are majorly dependent on the linker properties and thus the linker plays a major design role for the performance of LNPs (Zhi et al., 2018). Based on the linking bonds present, the lipids can be categorized into ether, ester, disulfide, phosphate, and other types. The approved cationic lipids, namely, DLin-MC3-DMA, ALC-0315, and SM-102, have bio-cleavable ester linkers which help dissociate the lipid-siRNA complex and thus the release of siRNA (Maier et al., 2013). The degraded fragments of the lipids are rapidly cleared from the body allowing for multiple doses within a short duration.

Lipidoids. Lipidoids are a novel class of lipid-like molecules resembling cationic lipids with alkylated tetraamine backbone. The clinically approved siRNA-based drug, Onpattro, is indeed based on lipidoids. The chemically synthesized lipidoids exhibit an extensive library of over 1,200 diverse lipids in one study with the potential in siRNA delivery for specific gene silencing purposes owing to pKa values between 6 and 7 (Dormenval et al., 2019). Khare *et al.* have explored the potential of lipidoid C12-200 in the formulation of a delivery vehicle owing to excellent knockdown efficacy and cellular uptake (Khare et al., 2022). The prepared lipidoid CS12-200 nanoparticles showed 83.8% siRNA loading when formulated with or without PEGylated helper lipids. The transfection efficacy for siRNA on neural cells was also increased twice without toxicity. Thus, the suitability of lipidoids for the delivery of siRNA for various purposes can be explored.

Liposomes

Liposomes are spherical vesicles composed of phospholipid bilayers with an aqueous core. These structures are advantageous due to their capability of carrying both hydrophilic siRNA molecules within the core and lipophilic drugs within the bilayers (Chadar et al., 2021). Liposomes can be prepared using mechanical dispersion methods such as ultrasonication, membrane extrusion, thin film hydration, and microfluidics (Al-Amin et al., 2020; Has and Sunthar, 2020; Jo et al., 2020; Zhang and Sun, 2021). Liposomes with sizes 100–1,000 nm are referred to as large unilamellar vesicles, while small unilamellar vesicle sizes range

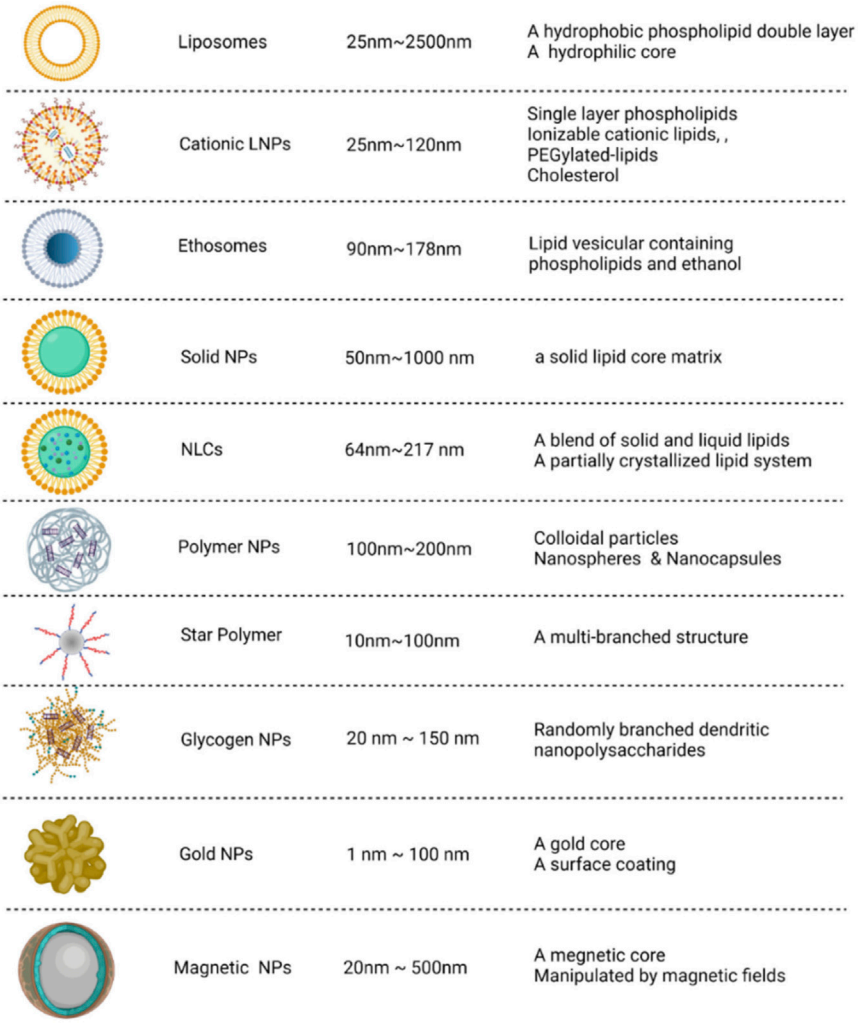

FIGURE 3
Potential nanocarrier configurations for siRNA delivery. The carriers can be categorized into organic (i.e., derived from lipid, polymeric and polysaccharides) and inorganic types. The size and structural features of these nanocarriers are depicted. Adapted from Ref. (Zhang et al., 2022a).

from 20–100 nm in size (Dymek and Sikora, 2022). Similar to all nanoparticles, the size and charge of liposomes determine the blood circulation time and cellular uptake rates (Ren et al., 2019; Lee et al., 2022). Nogueira *et al.* showed efficient siRNA delivery to activated macrophages using neutral lipid DOPE-based PEGylated liposomes (Nogueira et al., 2017), as shown in Figure 6. The DOPE-based liposomes with PEG exhibited almost neutral surface charge and thus showed a higher stealth degree, i.e. reducing the uptake by mononuclear phagocyte system. Further, the PEGylated liposomes anchored with folate targeted peptides showed high specific delivery of siRNA for gene silencing applications. In another aspect, the cationic liposomes showed more internalization efficiency than anionic liposomes due to their better interactions with the negatively charged plasma membrane. However, cationic liposomes may generate reactive oxygen species causing cytotoxicity (Kulkarni et al., 2018). Lechanteur *et al.* concluded that the cytotoxicity by cationic liposomes complexed with siRNA was dependent on the molar ratio of nitrogen on the IL to phosphate on RNA (N/P) and they can be safely used with the N/P ratio of 2.5 (Lechanteur et al., 2018).

Polymer nanoparticles

The application of polymers for siRNA delivery has been well explored. Common biodegradable polymers such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and poly (lactic-co-glycolide) (PLGA) are already approved by the Food and Drug Administration (FDA) for drug delivery applications. These polymers offer excellent biocompatibility and low immunogenicity (Wood, 2018; Saeed et al., 2021). Similar to lipids, surface charges of the resultant nanoparticles and the molecular weight of the constituting polymer influence siRNA delivery to the desired site. Cationic polymers such as polyethyleneimine and poly (l-lysine) have also been explored. However, these polymers show increased toxicity with increasing molecular weight. Karimov *et al.* have attempted the small linear polyethyleneimines modified with tyrosine for enhanced siRNA attachments (Karimov et al., 2021). The γ-[32P]-ATP labeled siRNA was efficiently transfected in three different xerographs without exhibiting toxicity. The knockdown efficacy in H441-luc cells due to the polymer-tyrosine-siRNA complex was also found efficient, proving the capabilities of polymeric nanoparticles for siRNA delivery.

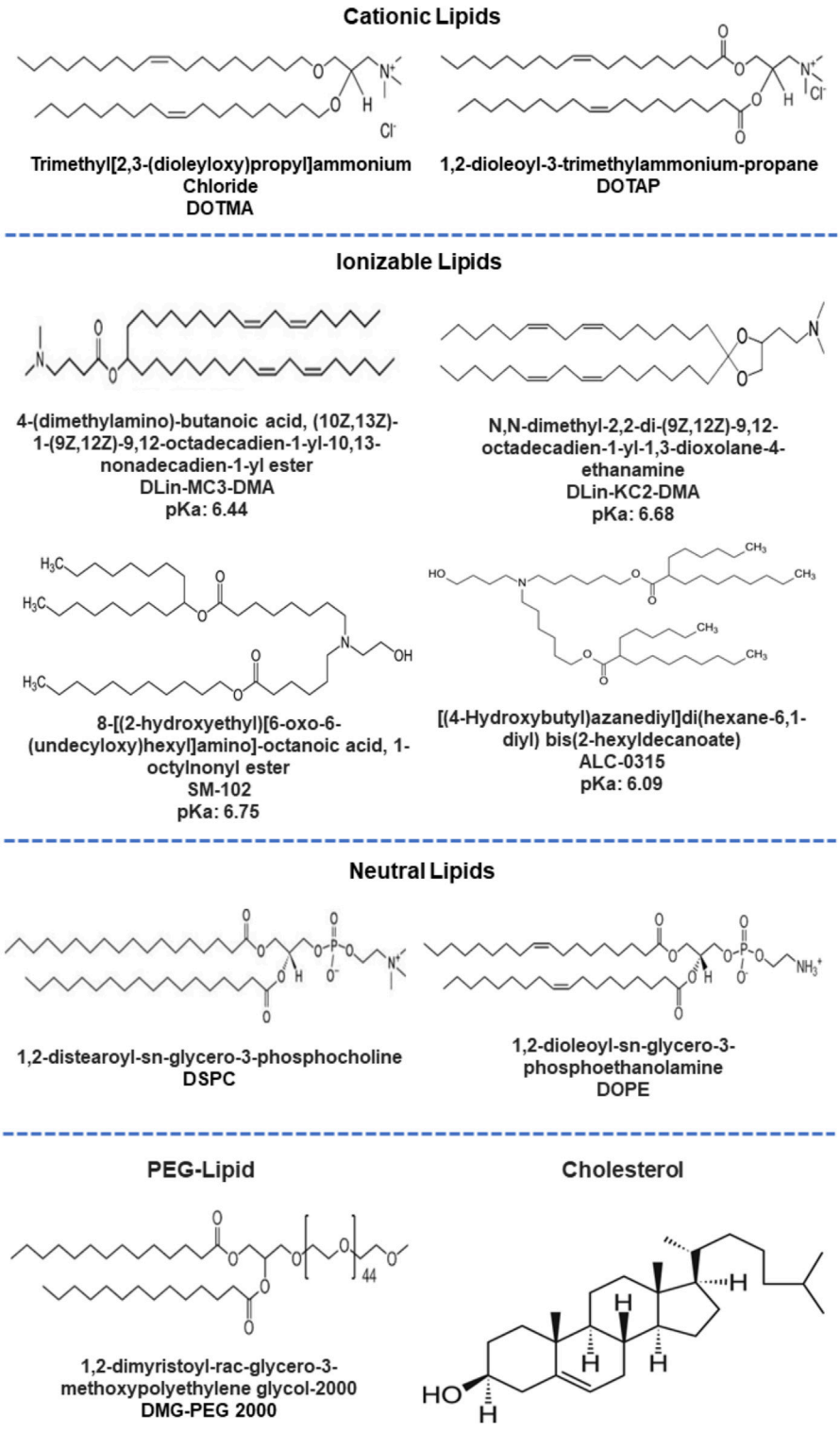

FIGURE 4
Different constituents (ILs, helper lipids, PEGylated lipids and cholesterol) used for lipid nanoformulations.

Further, studies have reported using low molecular weight polymer polyethyleneimine (PEI) successfully for siRNA carriers against breast cancer (Aliabadi et al., 2020; Uludağ et al., 2020). Also, the lipophilic PEIs was used for carrying the siRNA for the toxicity studies on human lung fibroblast cells and delivery of siRNA against Human Coronavirus 229E. The polymer siRNA complex showed more than 85% cell viability, and their transfection efficiency was similar to reference Lipofectamine™ (Montazeri Aliabadi et al., 2021). Further, biodegradable cationic polymers with ester bonds, such as poly (beta-amino ester), are used for siRNA delivery as they offer effective endosomal escape, flexible

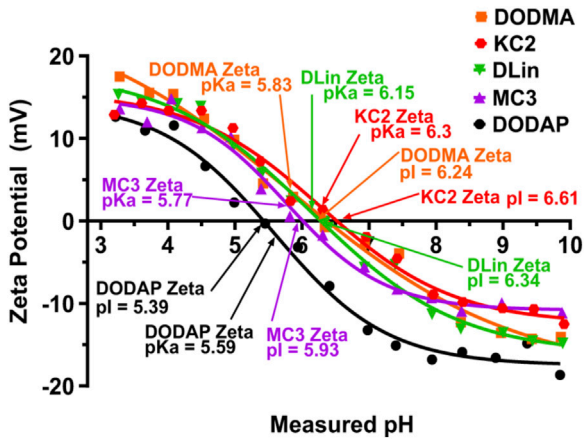

FIGURE 5
The ionization characteristics of the commercially available ILs obtained through zeta potential measurement. The LNPs showed a transition of charges-positive zeta potential at low pH and negative zeta potential at high pH, covering the range of endosomal and lysosomal pH. Adapted from Ref. (Carrasco et al., 2021).

conjugate binding capabilities, high stability, and tunable charge density (Nezhad, 2022).

Preparation of small interfering RNA-loaded lipid nanoparticles

The conventional preparation method for lipid nanoparticles, called thin film hydration, includes the dissolution of the constituent lipids in organic solvents followed by the gradual evaporation of the solvent to form a thin layer of dried lipids. The film is then rehydrated with a buffer containing the carrier molecules such as RNA or drugs (Zhang et al., 2021a). Another popular method for LNPs preparation includes the dissolution of a mixture of constituent lipids in ethanol and mixing it with the aqueous phase containing the load molecules such as siRNA (in phosphate buffers). The homogeneity of the mixing in such methods was inadequate leading to higher polydispersity index. With the advancement in instrumentation, microfluidic chambers mix these two solutions at a particular flow rate to obtain the desired size range of LNPs (Ly et al., 2022; Masatoshi et al., 2022; Younis et al., 2022). The obtained LNPs solution is further concentrated using dialysis and quantified for the extent of loading for nucleic acid molecules. Alternatively, polycarbonate-based membrane filters can prepare narrow-sized LNPs (Syama et al., 2022). Figure 7 depicts the steps involved in the preparation of LNPs using microfluidic method. The synthesis methods should critically consider some process parameters such as the molar ratio of lipid components, N/P ratio, and the flow rate of mixing (in the case of microfluidic chambers). The prepared LNPs can be qualitatively characterized through particle size distribution, structure of LNPs (as shown in Figure 3), surface charges and pH for their stability performance. While, siRNA encapsulation and transfection efficiency determine the efficacy/potency of prepared LNPs as therapeutics. The detailed perspective on the preparation of LNPs for siRNA delivery can be found elsewhere (Aldosari et al., 2021; Tenchov et al., 2021).

Stability of lipid nanoparticles

For effective therapeutic use of an LNP product, the formulation lipids should be rapidly metabolized *in vivo*, yet

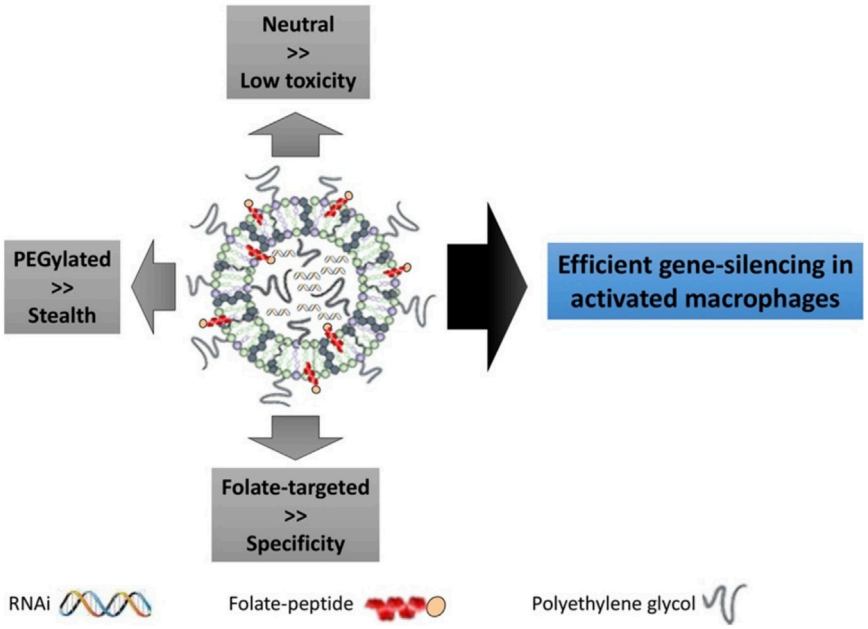

FIGURE 6
Folate incorporated liposome formulation for gene silencing applications. siRNA for Mcl-1 (a protein expressed in the rheumatoid joint macrophages) silencing was loaded in liposome and targeted towards folate receptor. PEGylated liposome offered stealth features against phagocytic uptake; neutral lipids exhibited low toxicity while specific targeting was observed by anchoring folate-targeted peptides. Adapted from Ref. (Nogueira et al., 2017).

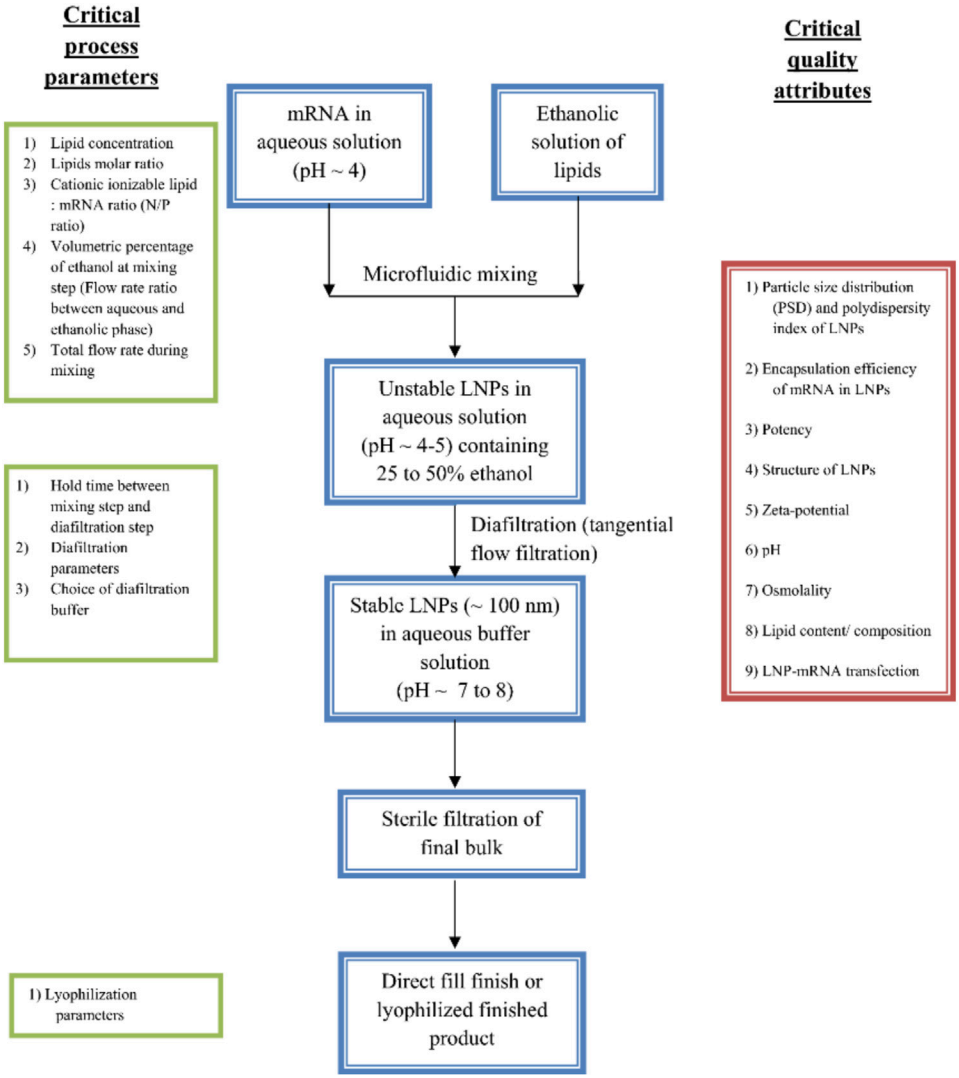

FIGURE 7
Microfluidics method for preparation of LNPs formulations. The preparation methods can be optimized through various process parameters such as ratio of constituent lipids, molar ratio of ILs and RNA (N/P ratio), the mixing flow rate and time, holding time, filtration and storage conditions. After the preparation, the LNPs can assess for their performance through qualitative analysis through size, charge and transfection and loading efficiency [adapted from (Ramachandran et al., 2022)].

exhibit good chemical stability in order to maintain sufficient shelf-life and initial circulation time. During the circulation period in extracellular fluids and intracellularly, several factors such as ionic strength, pH, adsorbing proteins and other environmental conditions may destabilize the LNPs, which can considerably affect the LNPs efficiency (Shah et al., 2022). Hence, stability of lipids under various conditions is an important aspect on protect the gene payloads, enable efficient delivery into target cells and assure functional outcomes *in vivo* (Koitabashi et al., 2021; Kon et al., 2022).

The ILs (Figure 8) are inspected frequently to find the ideal one for efficacy while maintaining muted toxicity profiles (Paramasivam et al., 2022). The elements of ILs, the ionizable head groups, linkers and the hydrocarbon tail chains, offer significant advantages in stability features (Albertsen et al., 2022). The mRNA-1273 LNP vaccine with ionizable amino-alcohol head group has pKa 6.75; the pKa range 6.2–6.6 was suggested to be optimal for protein expression following IV delivery, which has been consistent with effective mRNA-1273 use (Hassett et al., 2019). The linker between ionizable head group and the hydrocarbon chain contributes to head group pKa and LNPs endosomal escape potential (Maier et al., 2013). The hydrocarbon chain tails help LNPs by altering the endosomal escape, stability during storage and toxicity (Suzuki et al., 2017). The hydrophobic tails with unsaturation and symmetry contribute to LNP stability. For instance, branched hydrophobic chains can be advantageous in the context of endosomal escape by creating a cone shaped structure (Zhang et al., 2021b).

Size of the LNP is another important factor that plays a vital role in stability as well as pharmacokinetics. A study found that small-sized LNPs (<35 nm) showed a tremendous down in lipid packing, stability, ability for endosomal escape, and the cause appears to a higher amount protein being adsorbed on the surface of LNPs (Sato

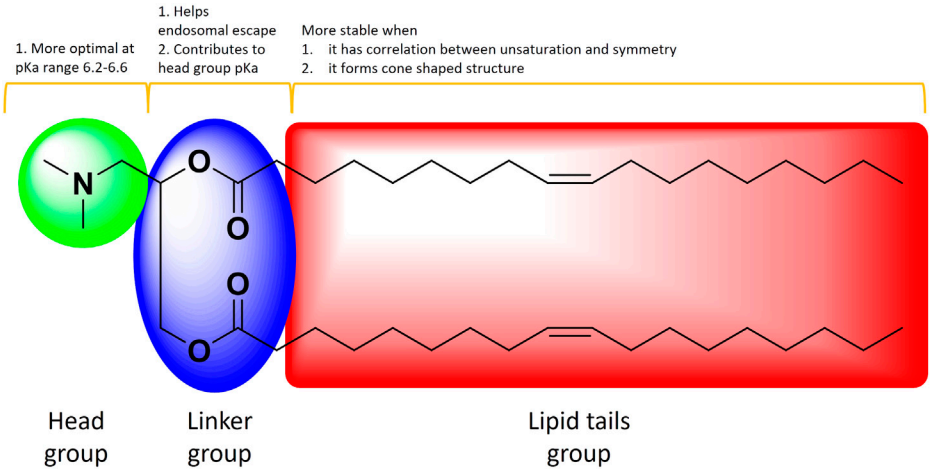

FIGURE 8
The schematic of an IL illustrating the main elements and how they contribute towards stability. Adapted from Ref (97)

et al., 2016) (Figure 9). Cabral et al., found only <50 nm nanoparticles can penetrate poorly permeable hypovascular tumors. Furthermore, increasing the permeability of hypovascular tumors using TGF-β signaling inhibitor improved the accumulation of >70 nm micelles, offering a way to enhance the efficacy of larger nanomedicines (Cabral et al., 2015). The stability of small LNPs in the blood circulation was increased by cholesterol a known helper lipid which increases the packing of lipids with unsaturated chains and therefore stabilize LNPs and avoid siRNA leakage (Hung et al., 2007).

Cleavage of lipid nanoparticles

The prime goal of developing degradable ILs is to improve lipid metabolism and prevent associated toxicities. The pharmacokinetic properties of ILs were strongly improved by introducing easily cleavable ester linkages into the tail hydrocarbon chain (Prata et al., 2004). Ester bonds is chemically stable under physiological conditions, but can be hydrolyzed by endogenous esterase or lipase in tissues and intracellular compartments (Luten et al., 2008). A summary of the hydrolytic cleavage pathway of lipids is shown in Figure 10. One or more ester linkages in the hydrophobic tail as well as the linker region appeared to be particularly interesting *in vivo* because of their on-demand degradation feature by esterases, which mitigate the pharmacokinetic properties of lipids with negligible toxicity (Sabnis et al., 2018). Owing to its ability to alter the head group pKa, the LNPs with ester linkage near to the head group suppresses the efficacy. In contrast, placing the ester linkage near the terminal ends of the lipid tail had little effect on the head group pKa and did not alter the *in vivo* outcome of the corresponding LNPs. LNPs formulated with the incorporation of ester linkages into the hydrocarbon chain region of the amino lipid preserved the head group linker structure with demonstrated efficacy (Gilham and Lehner, 2005). One study explored fully biodegradable ester bonds in the hydrophobic tails; the hydrolysis produced water-soluble alkanol amine that were readily eliminated from tissues, resulting in a reduced toxicity and liberation of endogenous oleic acids (Sato et al., 2019). Alternatively, replacing double bonds with ester linkages produces hydrolytic cleavage products that are quickly merged into catabolic pathways, without dropping capability. Multiple studies have shown such biodegradable ILs containing ester bonds attained instant elimination and excretion as well as significant permissibility in rodents and non-human primates after intravenous (Sato et al., 2019) and intramuscular (Hassett et al., 2019) administration. The fragmented lipids need to be metabolized in the plasma because these smaller fragments are often carried into systemic circulation. Hence, plasma stability is an applicable measure of overall biodegradability and potential for accumulation over time (Pei et al., 2022).

Targeted delivery of nano-formulations

The targeting of LNPs is crucial for the success of the treatment process. The conventional LNPs resemble the low-density lipids and thus can be adsorbed by Apolipoprotein E (ApoE) in the blood. These adsorbed LNPs typically accumulate in the liver and their hepatocytic uptake occurs *via* various lipoprotein receptors (Tian et al., 2019; Younis et al., 2022). Thus, targeting organs or tissues other than the liver is complex and inefficient by the conventional LNPs (Morán et al., 2022). Better understanding and control of the LNP fate *in vivo* is important and this has been actively explored in numerous studies.

Ionizable cationic lipids

Algarni *et al.* have explored the targeting efficiency of three ionizable cationic lipids, DLin-MC3-DMA, DLin-KC2-DMA and DODAP (1,2-dioleoyl-3-dimethylammonium-propane), for the organ-specific delivery of pDNA (Algarni et al., 2022). The intravenous administration in mice models with LNPs formulations

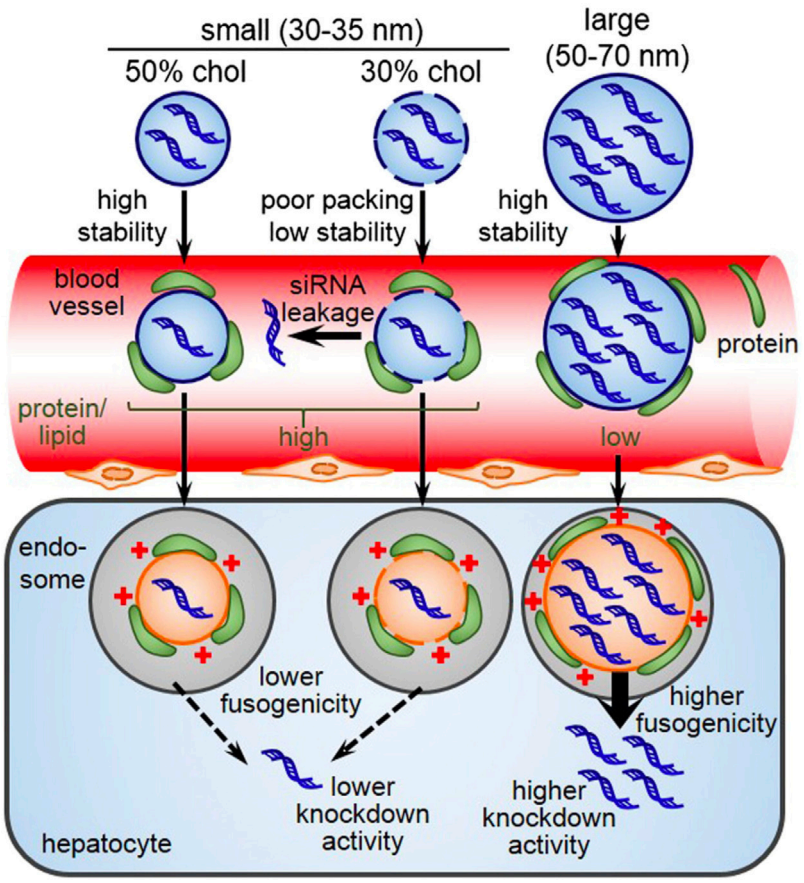

FIGURE 9
Schematic illustration of the stability of LNPs based on size. Adapted from Ref. (Sato et al., 2016).

showed that the DLin-MC3-DMA and DLin-KC2-DMA bearing LNPs more precisely and efficiently transfected the nucleic acid cargo to the spleen instead of the liver. The structure of ILs, DLin-MC3-DMA and DLin-KC2-DMA with two double bonds per alkyl chains, has influenced the transfection efficiency in the spleen. Thus, the selection of suitable ILs may improve the targeting.

Aptamer

Besides the difficulty of the naked siRNA passing through the cell membrane, pulmonary proteases, mucus layer, and macrophage-related inflammation also pose obstacles to siRNA delivery (Terada et al., 2021). Organ and site-directed targeting of siRNAs is also a significant challenge in the delivery and aptamers, which are highly specific single-stranded oligonucleotides directed against a target, could help in this approach (Khanali et al., 2021). For use in COVID-19 disease, aptamers prepared against the receptor binding domain (RBD) of the viral spike protein were prepared by the SELEX (Systemic Evolution of Ligands by EXponential enrichment) method and then conjugated with the siRNA bearing LNP conjugates (Saify Nabiabad et al., 2022) as shown in Figure 11. A study used 50–90 μM of aptamers to siRNA-LNP conjugate containing around 40–80 nM of siRNA and found ~50% inhibitory reduction *in vitro* in SARS-CoV-2 copies. This study also included a case study of a SARS-CoV-2 patient administered 10 mg of the aptamer-siRNA-LNP formulation by inhalation for 6 days, which indicated improvement in overall conditions as indicated by chest radiological and biochemical observations (Saify Nabiabad et al., 2022). The LNP contained DMKE (45%) (O,O′-dimyristyl-N-lysyl glutamate), DSPE-PEG2000 (4%), and cholesterol (46%), and was prepared by a methanol and chloroform mixture (2:1, v/v).

Many aptamers have been additionally developed for blocking the interaction of the S protein-ACE2 receptor, preventing viral entry (Gupta et al., 2021a; Li et al., 2021; Schmitz et al., 2021). Considering the high mutation rate of the spike protein, the search for a universally developed aptamer targeting all available variants is challenging. One such recent study designed an aptamer 'MSA52′from a library of specifically curated aptamers with K_d values of 2–10 nM targeting the wildtype, and B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.429 (Epsilon), B.1.617.2 (Delta), B.1.1.529 (Omicron) variants (Zhang et al., 2022b). However, the *in vitro* and *in vivo* targeting aspects of these aptamers are yet to be verified.

Selective organ targeting

Selective organ targeting (SORT) is a new technique for the regulated delivery of siRNA to specific targets with the help of

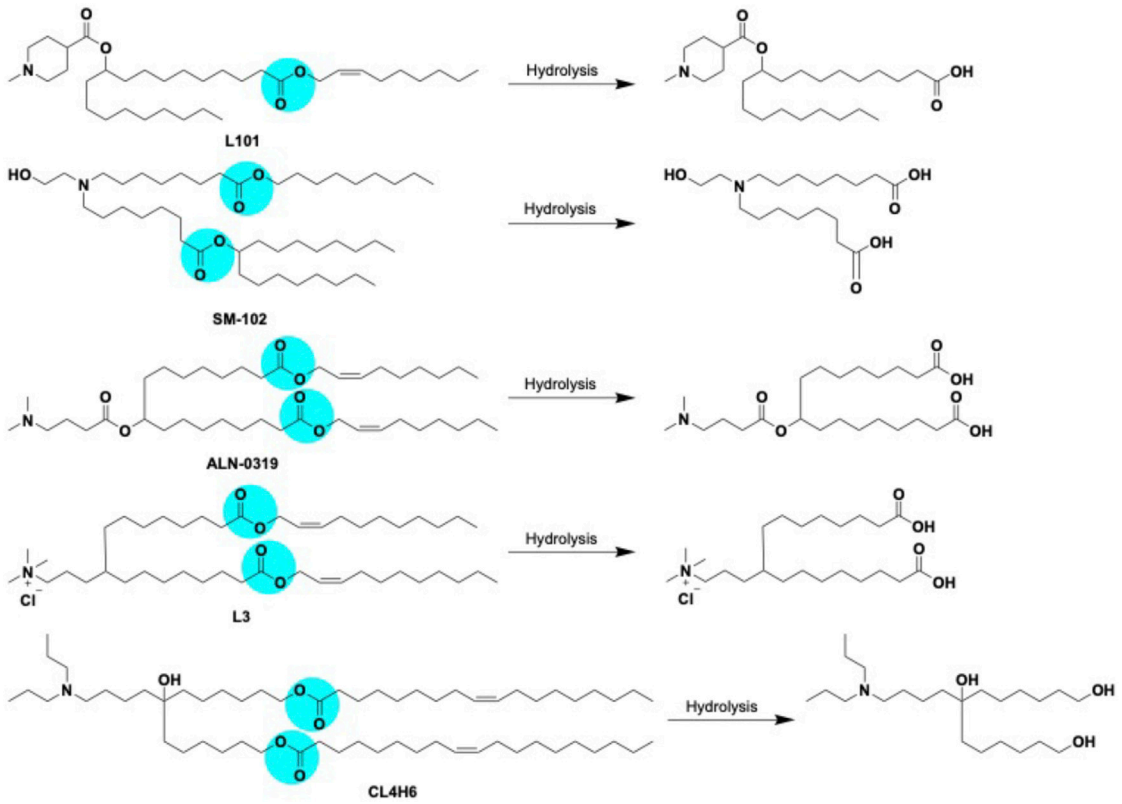

FIGURE 10
Biodegradable LNPs L101, SM-102, ALN-0319 (adapted from ref 113) L3 (adapted from ref 112) CL4H6 (adapted from ref 111) with cleavable ester linkage sites (highlighted in blue color) and expected hydrolysis pathway catalyzed by esterases.

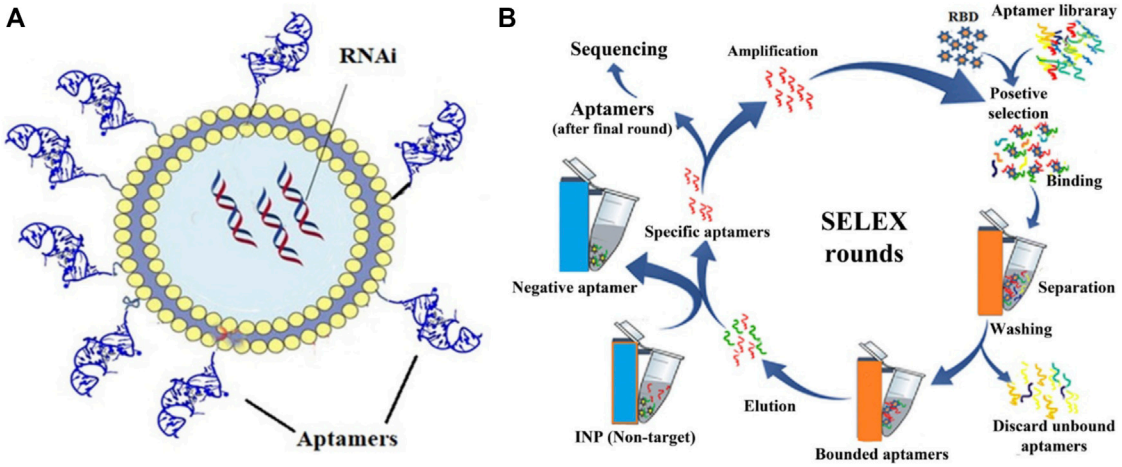

FIGURE 11
(A) The structure of aptamer molecules conjugated onto LNP-RNAi complex. LNPs were first complexed with siRNAs, which were then conjugated with synthesized aptamers. **(B)** SELEX-based method for aptamer selection after multiple rounds of binding, separation, washing, elution and amplification. Adapted from ref. (Saify Nabiabad et al., 2022).

LNPs. In conventional LNPs nanocarrier systems, the balance of ionizable groups and hydrophobicity of lipid nanoparticles determines the effective intracellular delivery. Such nanocarriers, may not show efficient targeting for various organs, except for liver. However, the SORT approach uses the unbalanced charge of lipids to alter the tissue tropism through the functional groups present and physiochemical properties of SORT molecules (Cheng et al., 2020). The design of the SORT includes introducing a fifth component into the formulation of LNPs without destabilizing the actual structure. The added SORT molecule controls the

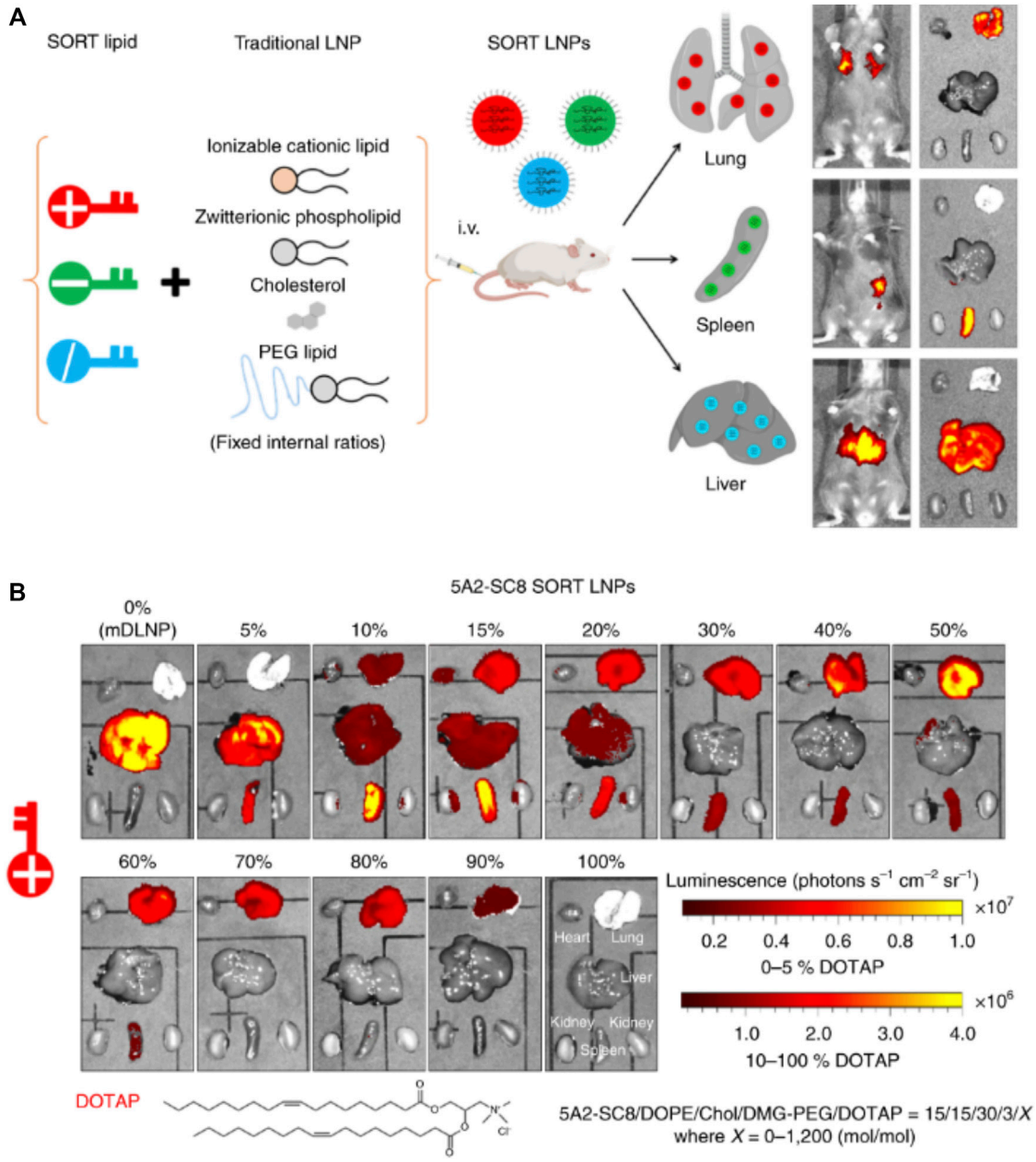

FIGURE 12
(A) Preparation of SORT LNPs for mRNA delivery for liver, spleen and lungs by incorporation of different SORT lipids in the formulation of LNPs. **(B)** The level of luciferase expression after administration of 5A2-SC8 SORT LNPs in mice models. With the increased cationic lipid DOTAP percentage, the expression of luciferase shifted from liver to spleen to lungs.

biodistribution, apparent pKa, and interaction with serum protein. The change in tissue tropism depends on surface charges of SORT molecules and their amount, which may help to predict the targeting of the LNPs for expression of load in particular organs (Wang et al., 2022). Compared to other targeting strategies such as aptamers or antibodies, SORT molecules offer innate targeting without any surface modifications. During the circulation, PEG-lipids of SORT-LNPs get desorbed, exposing the SORT molecules, followed by the adsorption of distinct serum proteins. These protein-adsorbed LNPs interact with specific receptors expressed by cells of the target organ (Cheng et al., 2020; Dilliard et al., 2021).

Cheng *et al.* used an engineered degradable dendrimer-based ionizable cationic lipid to target the lung, spleen, and liver. Here, the 5A2-SC8 SORT lipid fraction (0%–100%) was added to DOTAP. The shift in target from the liver to the spleen to the lungs was observed as an expression of luciferase protein, as shown in Figure 12. Another SORT molecule, 4A3-SC8, was used with 20% DODAP for liver targeting, 50% DOTAP for lung targeting, and 10% 18 PA for spleen targeting (Wang et al., 2022).

Effects of incorporation of SORT molecules in LNPs formulations visible from luminescence profile for tested mice models. Adapted from Ref. (Cheng et al., 2020).

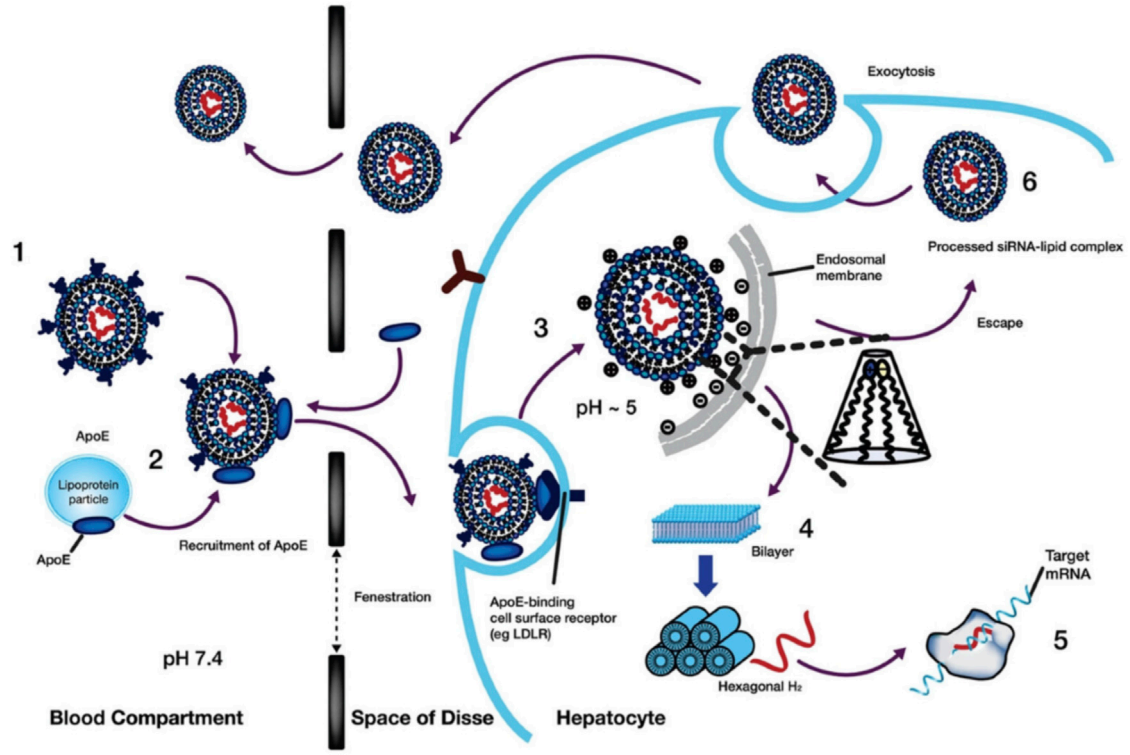

FIGURE 13
Internalization and release of RNA molecules in the hepatocytes. At physiological pH 7.4, the LNPs are adsorbed with ApoE protein which binds to low-density lipoprotein receptors in hepatic cells. The endocytic internalization of LNPs resulted in protonation of the surface due to acidic pH 5. The destabilization LNPs alters the hexagonal-like structure to release the loaded RNA molecules within the cell membrane for upregulation (mRNA) or downregulation (siRNA). The destabilized LNPs structure disintegrates and is removed while some LNPs face endosomal escape. Adapted from Ref. (Schlich et al., 2021).

Release of small interfering RNA

The internalization of LNPs occurs through the endocytosis process (Lee et al., 2022). In the case of hepatocytes, the serum proteins such as ApoE are hypothesized to adsorb on the LNPs surface and interact with lipoprotein receptors for cellular internalization (Morán et al., 2022), as shown in Figure 13 (Zhang et al., 2020). In other cases, antibodies specific to cell surface markers can mediate LNPs internalization (Kampel et al., 2021). With the maturation of the endosomes, the pH decreases below the pKa value, resulting in the increased protonation of the ILs (Younis et al., 2022). The accumulation of accumulation protons and counterions enhances the osmotic transportation of ions and water from the cytoplasm into the endosome. The rapid ionization at pKa values creates proton sponge effects resulting in osmotic swelling (Kalita et al., 2022). The electrostatic interaction between the cationic lipids forming LNPs, and anionic lipids in the plasma membrane can further destabilize the endosomal membrane. During the process, the planar endosomal bilayer structure rearranges to a hexagonal-like shape (Schlich et al., 2021), further bursting of endosomes releasing the siRNA in the cytoplasm following the complete disintegration of LNP structure during the process.

Current clinical status

Many modified formulations for siRNAs delivery have been demonstrated to have overcome the challenges with membrane penetration and stability. Several candidates for gene silencing are now in the clinical trial pipeline, with the major breakthrough occurring in 2018 with the FDA approval of the first siRNA therapeutics by Alnylam® Pharmaceuticals. The nanoparticle-based siRNA formulation, known as Patisiran (ONPATTRO™) targets transthyretin mRNA and is used for the treatment of polyneuropathy in a hereditary form of transthyretin-mediated (hATTR) amyloidosis (Wood, 2018). As indicated by (de Brito et al., 2022), many drug candidates are still in phase 3 trials. Out of which, Vutrisiran and Inclisiran have been recently approved. Vutrisiran, Givosiran (Givlaari®), Lumasiran (Oxluma®), and Inclisiran (Leqvio®), are the siRNA-based formulations employed to treat hATTR-related polyneuropathy, acute hepatic porphyria, primary hyperoxaluria, and atherosclerotic cardiovascular disorders, respectively (Balwani et al., 2020; Raal et al., 2020; Garrelfs et al., 2021; Aimo et al., 2022). Table 2 summarizes the siRNA formulations under various clinical trials for diverse disorders. No clinical research has now been focused on the siRNA-mediated gene silencing in SARS-CoV-2, but a similar approach can be followed to target divergent sections of the genome of COVID-19 virus leading to the degradation of the viral mRNA sequence. Considering the target specificity and lower side effects, siRNA therapeutics have emerged as a promising therapeutic class (Forgham et al., 2022). However, the fast mutation rate of the viral genome has to be kept in mind with potentially targeting the conserved viral

TABLE 2 Approved and under trial siRNA therapeutics for different diseases.

Name	Manufacturer	Disease	mRNA target	Delivery system	DrugBank accession number	Ref
FDA-approved siRNA therapeutics						
Patisiran (Onpattro®)	Alnylam	Polyneuropathy in hereditary transthyretin-mediated amyloidosis	Transthyretin (TTR)	Lipid nanoparticle (DLin-MC3-DMA)	DB14582	Wood, (2018)
Lumasiran (Oxluma®)	Alnylam	Primary hyperoxaluria type 1	Hydroxyacid oxidase 1 (HAO1)	GalNAc	DB15935	Garrelfs et al. (2021)
Givosiran (Givlaari®)	Alnylam	Acute hepatic porphyria	Aminolevulinic acid synthase 1 (ALAS-1)	GalNAc	DB15066	Balwani et al. (2020)
Inclisiran (Leqvio®)	Alnylam-Novartis	Atherosclerotic cardiovascular disease	Proprotein convertase subtilisin/kexin type 9 (PCSK9)	GalNAc	DB14901	Raal et al. (2020)
Vutrisiran (Amvuttra®)	Alnylam	Hereditary transthyretin mediated amyloidosis	Transthyretin (TTR)	GalNAc	DB16699	Adams et al. (2022); Aimo et al. (2022)
siRNA therapeutics under clinical trial						
Teprasiran (QPI-1002)	Quark-Norvartis	Prevention of Major Adverse Kidney Events (MAKE)	p53	Naked siRNA molecule	DB15064	Thielmann et al. (2021)
Fitusiran (ALN-AT3)	Alnylam-Sanofi Genzyme	Haemophilia A & B	Antithrombin (AT)	GalNAc	DB15002	Srivastava et al. (2021)
Nedosiran (PHYOX1)	Dicerna-Alnylam	Acute kidney injury	Hepatic lactate dehydrogenase (LDH)	GalNAc	-	Liu et al. (2022)
Cosdosiran (QPI-1007)	Quark	Non-arteritic anterior ischemic optic neuropathy (NAION)	Caspase-2	Naked siRNA molecule	-	Jiang et al. (2021)
Tivanisiran (SYL1001)	Sylentis	Dry eye disease (DED)	Transient Receptor Potential Vanilloid 1 (TRPV1)	Naked siRNA molecule	-	Moreno-Montañés et al. (2018)
Fazirsiran (ARO-AAT/ TAK 999)	Arrowhead Pharmaceuticals and Takeda	Liver disease associated with α-1 antitrypsin deficiency (AATD)	Mutant α-1 antitrypsin (Z-AAT)	GalNAc	-	Strnad et al. (2022) (NCT03945292)
ARO-APOC3	Arrowhead Pharmaceuticals	Familial chylomicronemia syndrome (FCS), and Severe Hypertriglyceridemia	Apolipoprotein C-III (APOC3)	GalNAc	-	Watts et al. (2020); Akoumianakis et al. (2021) (NCT05089084)(NCT04720534)
ARO-ANG3	Arrowhead Pharmaceuticals	Treatment of Homozygous Familial Hypercholesterolemia (HOFH), and Mixed Dyslipidemia	Angiopoietin-like 3 (ANGPTL3)	GalNAc	-	Watts et al. (2020); O'Donoghue et al. (2022) (NCT05217667)(NCT04832971)
Olpasiran (AMG 890)	Amgen Pharmaceuticals	Atherosclerotic cardiovascular diseases (ASCVD)	Lipoprotein(a) (Lp(a))	GalNAc	-	O'Donoghue et al. (2022) (NCT05581303)
Revusiran (ALN-TTRSC)	Alnylam	Polyneuropathy in hereditary transthyretin-mediated amyloidosis	Transthyretin (TTR)	GalNAc	DB16309	Judge et al. (2020) (NCT02319005)
SLN360	Silence Therapeutics	Atherosclerotic cardiovascular diseases (ASCVD)	Lipoprotein(a) (Lp(a))	GalNAc	-	Rider et al. (2022) (NCT05537571)
SLN124	Silence Therapeutics	Erythropoiesis and hepatic iron-overload	Transmembrane serine protease 6 (TMPRSS6)	GalNAc	-	Vadolas et al. (2021) (NCT04718844)
ALN-APP	Alnylam-Regeneron	Early onset Alzheimer's disease and cerebral amyloid angiopathy (CAA)	Amyloid precursor protein (APP)	C16 conjugate technology	-	Akoumianakis et al. (2021) (NCT05231785)
Cemdisiran (ALN-CC5)	Alnylam-Regeneron	Complement-mediated diseases, *viz.*, paroxysmal nocturnal hemoglobinuria (PNH), and immunoglobulin A nephropathy (IgAN)	Complement component 5 (C5)	GalNAc	DB16121	Badri et al. (2021) (NCT03841448)
ALN-HBV02/VIR-2218	Alnylam-Vir Biotechnology	Chronic hepatitis B virus (HBV) infection	All viral transcripts and HBV protein	GalNAc and Enhanced stabilization chemistry plus (ESC+) technology	-	Gupta et al. (2021b) (NCT03672188)

(Continued on following page)

TABLE 2 (*Continued*) Approved and under trial siRNA therapeutics for different diseases.

Name	Manufacturer	Disease	mRNA target	Delivery system	DrugBank accession number	Ref
ALN-XDH	Alnylam	Gout	Xanthine dehydrogenase (XDH)	GalNAc and Enhanced stabilization chemistry plus (ESC+) technology	-	(NCT05256810)
Zilebesiran (ALN-AGT)	Alnylam	Hypertension	Angiotensinogen	GalNAc and Enhanced stabilization chemistry plus (ESC+) technology	-	(NCT04936035)
ALN-KHK	Alnylam	Type-2 Diabetes Mellitus (T2DM)	ketohexokinase (KHK) or fructokinase	GalNAc and Enhanced stabilization chemistry plus (ESC+) technology	-	NA
Belcesiran (DCR-A1AT)	Dicerna-Alnylam	α-1 antitrypsin (AAT) deficiency-associated liver disease (AATLD)	α-1 antitrypsin (AAT)	GalXC™ RNAi platform based on GalNAc	-	(NCT04764448)
RG6346 (DCR-HBVS)	Dicerna-Roche	Chronic Hepatitis B Virus (HBV) Infection	Conserved S region for the treatment of HBV	GalXC™ RNAi platform based on GalNAc	-	(NCT03772249)(NCT04225715)
DCR-AUD	Dicerna-Alnylam	Alcohol use disorder (AUD)	Aldehyde dehydrogenase 2 family (ALDH2)	GalXC™ RNAi platform based on GalNAc	-	Sasso et al. (2022) (NCT05021640)
AB-729	Arbutus Biopharma	Chronic Hepatitis B Infection	Hepatocytes	GalNAc	-	Phillips et al. (2022) (NCT04980482)
STP705	Sirnaomics	Keloid scarring	Transforming growth factor beta 1 (TGF-β1)/cyclooxygenase-2 (COX-2)	Polypeptide Nanoparticle (PNP)	-	Zhou et al. (2017) (NCT04844840)
ALN-HSD	Alnylam-Regeneron	Non-alcoholic steatohepatitis (NASH)	Hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13)	GalNAc and Enhanced stabilization chemistry plus (ESC+) technology	-	Cui et al. (2021) (NCT04565717)
siG-12D-LODER	Silenseed	Pancreatic Cancer	KRAS (Kirsten rat sarcoma virus)	-	-	Golan et al. (2015) (NCT01676259)
GSK 4532990 (ARO-HSD)	GlaxoSmithKline/ Arrowhead Pharmaceuticals	Non-alcoholic steatohepatitis (NASH)	Hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13)	GalNAc	-	(NCT05583344)(NCT04202354)

ClinicalTrials.gov Identifier: NCTXXXXXXXX.

domains with the help of available libraries and computational resources.

Conclusion

Considering the ever-changing dynamic mutations in the SARS-CoV-2 genome, the demand for well-targeted specific and highly effective therapeutics are needed. Genomic regions of the virus conserved across the variants and sub-variants could be first targeted to inhibit viral entry and replication in the host. Many *in silico* algorithms and web servers have been deployed to design siRNAs following the three golden rules of design, that is Ui-Tei, Amarzguioui and Reynolds. The accessory parameters such as heat capacity (C_P), melting temperature (T_m), GC content, and off-target matches are predicted computationally. In a limited set of studies, some validation efforts have been attempted to experimentally verify the silencing efficiency of the computationally designed siRNAs, but this will require more extensive studies to assure confidence in the theoretical designs. Nevertheless, a handful of effective designs are now available that could be tested in a clinical setting, should there be sufficient impetus from clinicians and industrial parties. Further, the designed siRNAs can be formulated with different nanocarriers for practical utility. Lipids-based and polymeric nanoparticles offer the flexibility of conjugation with siRNAs and surface functionalization for targeted delivery. siRNAs-based therapies have been approved for other diseases, but in the case of COVID-19 clinical trials are yet to be undertaken. Along with the efficacy of nano-formulations, other inflammatory responses should be investigated through *in vivo* studies. The COVID-19 treatment, in addition to targeting the viral cause, can be directed to aberrant, excessive inflammation that is ultimately cause of patient exhaustion in clinic. The dosage of siRNAs should be confined to a minimum concentration, preferably below 100 nM at local sites and <1 mg/kg overall for practical translation of delivery systems. The siRNA-based nano-

formulations appear to be promising for the therapy of contagious COVID-19 and post-COVID inflammations.

Author contributions

RF, CP, AR, and LP drafted the manuscript and LP and HU edited the manuscript.

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Conflict of interest

HU has ownership interest in materials that could be used in siRNA delivery. AR was supported by a MITACS fellowship sponsored by RJH Biosciences.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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PVC containing silver nanoparticles with antimicrobial properties effective against SARS-CoV-2

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Poly (vinyl chloride) (PVC) is commonly used to manufacture biomedical devices and hospital components, but it does not present antimicrobial activity enough to prevent biofouling. With the emergence of new microorganisms and viruses, such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that was responsible for the global pandemic caused by Coronavirus Disease 2019 (COVID-19), it is evident the importance of the development of self-disinfectant PVC for hospital environments and medical clinics where infected people remain for a long time. In this contribution, PVC nanocomposites with silver nanoparticles (AgNPs) were prepared in the molten state. AgNPs are well-known as antimicrobial agents suitable for designing antimicrobial polymer nanocomposites. Adding 0.1 to 0.5 wt% AgNPs significantly reduced Young's modulus and ultimate tensile strength of PVC due to the emergence of microstructural defects in the PVC/AgNP nanocomposites, but the impact strength did not change significantly. Furthermore, nanocomposites have a higher yellowness index (YI) and lower optical bandgap values than PVC. The PVC/AgNP nanocomposites present virucidal activity against SARS-CoV-2 (B.1.1.28 strain) within 48 h when the AgNP content is at least 0.3 wt%, suitable for manufacturing furniture and hospital equipment with self-disinfectant capacity to avoid secondary routes of COVID-19 contagion.

KEYWORDS

SARS-CoV-2, poly(vinyl chloride), COVID-19, silver, nanocomposites

1 Introduction

Poly (vinyl chloride) (PVC) has been used in the medical field for over three decades, widely applied in medical applications today because it is impervious to germs, easily cleaned, and allows sterilization and disposable applications that reduce healthcare infections (Zhao and Courtney, 2009; Wypych, 2016). In addition to applications in the biomedical sector, PVC has been used in bottles, cables, domestic appliances, pipes, food contact films, among others (Schiller, 2015). The reasons for the extensive technological applications of PVC, including healthcare and packaging industry, is also due to the unique

combination of properties, just to name a few, flexibility, transparency, chemical stability, biocompatibility and resilience, ease of processing, accessibility (cost and marketing), and recyclability.

Stabilizers and processing additives are indispensable to prevent the degradation of PVC during its thermal processing to guarantee the confection of biomedical devices and structural parts with suitable mechanical performance. Pristine PVC can present bacteriostatic activity against some bacteria (Zhao and Courtney, 2009; Schiller, 2015). However, stabilized and plasticized PVC does not present enough antimicrobial properties to impede biofilm formation since phthalate ester plasticizers, crazing, and other surface defects from UV exposure make additive PVC more susceptible to biofouling (Mark, 2004). Then, it is necessary to apply biocide additives in PVC formulations because it is vulnerable to biofilm formation due to the growth of multilayer bacterial colonies covered by an extracellular matrix composed mainly of polysaccharides (Ferreira et al., 2015).

Although controversies and doubts about the effects occasioned by stabilizers and processing additives on human health along with short and long-term exposure times, PVC has been considered an excellent material with biocompatibility, chemical stability, and sterilization resistance combined with economic advances that make this polymer one of the main materials used in the manufacture of products of extreme importance in medicine, such as flexible blood containers, urine ostomy bags, flexible tubes, inhalation masks, oxygen masks, and personal protective equipment (Zhong et al., 2013; Lewandowski and Skórczewska, 2022).

Since 2019, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and its variants have caused a system collapse and brought about health systems and health crises in several countries (Tao et al., 2021). SARS-CoV-2 and variants are highly contagious viruses transmitted between humans mainly through respiratory droplets *via* aerosol (Howard et al., 2020; Li et al., 2020). It has been alarmed that SARS-CoV-2 can maintain its potential for contagion even after 24 h on the surface of polymeric materials (van Doremalen et al., 2020). Therefore, PVC with self-disinfecting capacity is relevant to produce structural components and products, such as handrails and wall guards, to prevent critical epidemiological issues in hospital environments and medical clinics (Balagna et al., 2020; Hasan et al., 2020).

The main procedures to confer auto-disinfectant properties to PVC are surface modification and mixing inorganic materials with intrinsic bactericidal and fungicidal properties (Behboudi et al., 2018). The blending with cationic polymers and functionalization with cationic groups (cationization) are other viable technological approaches to add bactericidal properties to PVC (Palencia et al., 2019). The incorporation of antimicrobial agents in the polymer matrix may bring some advantages over other methods, such as the possibility of using conventional polymer processing equipment (extruders, injectors, among others) and longer time extension of the antimicrobial activity over time. However, the development of composites by this route generally requires high amounts of antimicrobial agents to achieve a bactericidal effect and not just a bacteriostatic activity. The development of polymer nanocomposites by using antimicrobial agents in the nanoscale can be an alternative way to avoid this problem. In concern of COVID-19 spreading, such technological approaches to develop self-sanitizing PVC are suitable to avoid the secondary routes of COVID-19 contagion, mainly in hospitals and healthcare clinics that involve touching a contaminated surface and then contamination with dirty hands as extensively reviewed in the literature (Marquès and Domingo, 2021; Correia et al., 2022).

Silver (Ag), copper (Cu), TiO_2, ZnO, Cu_2O, and CuO are the main inorganic antimicrobial agents applied for the development of antimicrobial materials (Sedighi et al., 2014; Gold et al., 2018; Vodnar et al., 2020). They act mainly by generating reactive oxygen species (ROS) and releasing metal ions that cause irreversible damage to biological components present in the viral structure and bacterial and fungal cells (Tan et al., 2019; Zhou et al., 2020). Several authors have shown the outstanding antimicrobial activity of silver nanoparticles (AgNPs) or silver-based nanoparticles over the other antimicrobial agents in polymeric nanocomposites (Pongnop et al., 2011; Narayanan and Han, 2017; Oliani et al., 2017; Shah et al., 2018; Kraśniewska et al., 2020; Morais et al., 2020; Rahimi et al., 2020), including against SARS-CoV-2 (Assis et al., 2021). A few studies have shown the auto-disinfectant ability of PVC/AgNP nanocomposites (Zampino et al., 2011; Azlin-Hasim et al., 2016; El-Sayed et al., 2016; Braga et al., 2018), but their antiviral capability against SARS-CoV-2 has not been investigated. Furthermore, it is important to mention that most of these works were not carried out by mixing PVC and AgNP in the molten state (Azlin-Hasim et al., 2016; El-Sayed et al., 2016; Braga et al., 2018). Generally, PVC/AgNP nanocomposites are prepared by solvent methods (typically casting and AgNP synthesis in the presence of dissolved PVC) that are not suitable for obtaining large products on an industrial scale. This contribution aims to fill this gap in the literature. Moreover, we evaluated the thermal stability, and mechanical properties of the PVC/AgNP nanocomposites.

2 Materials and methods

2.1 Materials

A rigid PVC compound in the form of pellets was supplied by Karina Plásticos (Brazil). AgNP liquid suspension (NpAg-925ETG) was purchased from TechNano Solutions (TNS, Brazil). HNO_3 (65%), $AgNO_3$ (99%), KSCN (>99%), $Zn(NO_3)_2{\cdot}6H_2O$ (96%–103%), $Cu(NO_3)_2{\cdot}3H_2O$ (98%–102%), and $Fe(NO_3)_3{\cdot}9H_2O$ (≥99.95%) were purchased from Synth (Brazil). All reagents were used as purchased without prior purification.

2.2 Methods

2.2.1 Preparation of the PVC/AgNP nanocomposites

The PVC and PVC/AgNP nanocomposites were prepared through melt processing in an internal mixer (Model 50EHT 3Z, Brabender GmBh & Co. KG, Germany) at 160°C and a rotor speed of 60 rpm. First, PVC (50 g) was plasticized for 2 min, and then the AgNP suspension was added (0.5, 1, and 2 mL). The PVC samples were mixed for 8–10 min, using a fill factor of 80%. The nanocomposites were named PVC/*X*AgNP, where *X* corresponds

to the AgNP content (0, 0.1, 0.3, and 0.5 wt%). The AgNP concentrations were estimated from the metal content measurements using Inductively Coupled Plasma Atomic Emission Optical Spectroscopy (ICP-OES).

Samples for impact testing were injected at 180 °C (test specimen dimensions according to ASTM D256 in a microinjection molder (Model 12cc, XPlore Instruments BV, The Netherlands), with mold temperature at 40 °C and 9 bar of pressure. The tensile samples were pressed in a hydraulic press (model SL 11, Solab Científica, Brazil) using a mold at 190°C, a residence time of 3 min, followed by 6 tons of pressure for 5 min. Then, the films (thickness = 1 mm) were wedge-cut in the specimen shapes following ASTM D1708.

2.3 Characterization

2.3.1 AgNP suspension

2.3.1.1 Dynamic light scattering (DLS)

The AgNP hydrodynamic diameter was characterized by dynamic light scattering (DLS), with a stable 90° scattering angle, using a Zetasizer Nano-ZS (Malvern Panalytical Ltd., Malvern, UK). The AgNP liquid suspension (50 µL) was diluted in distilled water (2 mL) before the DLS measurements.

2.3.1.2 Zeta potential (ζ)

The Zeta potential (ζ) was calculated with the Smoluchowski model using electrophoretic mobility measurements of the nanoparticles obtained by Zetasizer Nano-ZS (Malvern Instruments, UK). The reading time to measure the Zeta potential data was 10 s, and the measurements were performed in duplicate.

2.3.1.3 Energy-dispersive X-ray spectroscopy (EDS)

EDS spectra were obtained using a JEOL compact scanning electron microscope (JSM-6010LA) using the secondary electron detector (SEI). The AgNP suspension (~20 µL) was previously deposited on carbon tape and then dried on a heating plate (300°C) in the ambient atmosphere.

2.3.1.4 Fourier-transform infrared absorption spectroscopy (FTIR)

Fourier-transform infrared absorption spectroscopy (FTIR) measurements were performed on a Thermo IS5 Nicolet spectrometer, using an attenuated total reflectance (ATR) accessory (ZnSe crystal). Spectral data acquisition was conducted in the range of 600–4,000 cm^{-1}, using 32 scans and a spectral resolution of 2 cm^{-1}. Before FTIR measurements, the AgNP suspension was previously deposited (2 drops) on KBr pellets and dried at 100 °C for 30 min in a vacuum oven (Vacuoterm).

2.3.1.5 Ultraviolet-visible absorption spectroscopy (UV-Vis spectroscopy)

UV-Vis spectroscopy measurements were performed using a UV-Vis spectrophotometer (Varian Cary, Model 50). The AgNP suspension was diluted in distilled water, and then the UV-Vis spectrum was collected.

2.3.1.6 Inductively Coupled Plasma Atomic Emission Optical Spectroscopy (ICP-OES)

The silver, zinc, and copper content in the AgNP suspension was quantitatively estimated by ICP-OES analysis. The measurements were performed in an equipment ICP-OES Axial View, model 710 Series (Varian). The instrumental conditions are detailed in (Supplementary Table S1). The calibration curve was prepared from $AgNO_3$, $Cu(NO_3)_2$, and $Zn(NO_3)_2$ aqueous solutions (HNO_3-3%).

2.3.2 PVC/AgNP nanocomposites

2.3.2.1 Scanning electron microscopy (SEM)

The samples with PVC were coated with a 20 nm thick gold layer, using Leica EM ACE 200 Sputter Coater (Leica Microsystems, Wetzlar, Germany). Micrographs were taken in a microscope FEI Quanta 250 (Thermo Fisher Scientific, Hillsboro, Oregon, United States), using an accelerating voltage of 10 kV, a spot size of 4 nm, and a magnification of 5,000x.

2.3.2.2 UV-Vis diffusive reflectance spectroscopy

The diffuse reflectance (R_d) spectra were collected in a UV-Vis spectrophotometer (Model Evolution 220, ThermoFisher, United States). Spectralon diffuse reflectance material based on polytetrafluoroethylene (PTFE) was applied as a white reflection pattern (reflection = 100%). These measurements were made in the range of 200–1000 nm with a spectral resolution of 1 nm. The yellowness index (YI) was calculated from the reflectance measurements by Eq. 1.

$$YI = \frac{(R+G)}{B^2} \tag{1}$$

where R, G, and B are reflectance intensity at 680, 530, and 470 nm, respectively.

The optical bandgaps (E_g) of the PVC samples were estimated from R_d data (in %) using Tauc's plots $[h\nu\ F\ (R_d)]^{1/n}$ *versus* hν and extrapolating the linear region in the radiation energy axis (hν). h is Planck's constant, ν is the frequency of electromagnetic radiation, n depends on the nature of the electronic transition (n is equal to two for indirect transition and to ½ for direct transition), and F (R_d) is the Kubelka-Munk function is determined given by Eq. 2 (Li et al., 2012; Shebi and Lisa, 2019).

$$F(R_d) = \frac{(100 - R_d)^2}{2R_d} \tag{2}$$

2.3.2.3 Fourier-transform infrared absorption spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) with attenuated total reflectance (ATR) diamond accessory was performed on Spectrum Two equipment (PerkinElmer Inc., Massachusetts, United States). The spectra were collected with 4 cm^{-1} spectral resolution, 64 scans, from 4,000 to 500 cm^{-1}. The PVC degradation was evaluated by the carbonyl ($I_{C=O}$), polyene ($I_{C=C}$), and hydroxyl (I_{OH}) indexes using Eq. 3, according to the literature (Yousif et al., 2016).

$$I = \frac{A_{group}}{A_{1328}} \tag{3}$$

where A_{1328} is the infrared absorbance reference peak at 1328 cm^{-1} associated with the scissoring and bending of CH_2 groups. A_{group} is the infrared absorption at 1722 (carbonyl group), 1602 (polyene), and 3,500 cm^{-1} (hydroxyl group) connected with chemical groups generated by the PVC degradation reactions.

2.3.2.4 Thermogravimetric analysis (TGA)

The thermal stability of the polymeric samples was evaluated by a TGA thermal analyzer (Mettler Toledo, United States) using alumina pans. The samples were heated from 50°C to 600°C (heating rate = 10°C min^{-1}) under N_2 atmosphere (50 mL min^{-1}).

2.3.2.5 X-ray photoelectron spectroscopy (XPS)

The XPS high-resolution spectra were collected using K-alpha + equipment (ThermoFisher Scientific Inc., Massachusetts, United States) with monochromatic radiation A1Kα at room temperature (pass energy = 50 keV; energy step = 0.1 eV). The samples were plasma etched to perform XPS depth-profile of silver and carbon elements (ion energy = 2000 eV; raster size = 2.00 mm; depth-profile etch time = 5 s). The etched depths of the PVC samples were estimated by the etching rate of Ta_2O_5 standard (0.29 nm s^{-1}). The XPS spectra peak-fittings were performed in CasaXPS version 2.3.25, using U 2 Tougaard background approximation and finite Lorentzian asymmetric (LF) lineshape (with relative sensitivity factors = 1). XPS spectra were calibrated to give C-C/C-H binding energy (C1s region) of 284.8 eV (Baibarac et al., 2021).

2.3.2.6 Mechanical properties

Uniaxial tensile tests were performed in a Universal Testing Machine from Instron, using a load cell of 50 kN and a test speed of 1.5 mm min^{-1}, according to ASTM D1708 (micro tensile). Notched Izod impact strength was measured at room temperature (25°C) by an Izod Impact Tester (Shanta Engineering, India) with a hammer pendulum of 2.71 J, following method A in ASTM 256D. All mechanical data were determined using 2-6 specimens.

2.3.2.7 Antiviral assays

Surface antiviral tests were carried out in triplicate according to the ISO 21702:2019 standard. Films were cut into 5 cm^2 squares, in laminar flow with sterile scissors, decontaminated with 70% ethanol, packed in surgical grade paper, sterilized for 20 min at 121°C in saturated steam under a pressure of 110 kPa (autoclave), and then dried in an oven at 51°C for 4 h.

Briefly, the Vero E6 cell line (ATCC–CRL1586) was cultured using Eagle's Minimal Essential Medium (EMEM) (Sigma-Aldrich) containing 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco®) incubated with 5% CO_2 at 37°C. After culturing, the cells were transferred to a 96-well plate containing 1×10^5 cells/well and incubated until reaching 80%–90% confluence. The virus inoculum used was SARS-CoV-2 (B.1.1.28 strain) 2.5×10^6 $TCID_{50}$/mL titrated according to $TCID_{50}$ (50% Tissue Culture Infectious Doses) method. For sample contamination, the tests were carried out in a BSL-3, in a biological safety cabinet Class II B2. 100 µL of the virus inoculum were added to the center of the samples, spread with a sterile disposable loop, and incubated at room temperature (direct contact times = 30, 60, and 120 min). The material was recovered with a sterile swab and added to a Falcon tube with 0.9 mL of EMEM medium, being vortexed for 1 min 150 µL of eluate aliquots were plated on previously 80%–90% confluent VeroE6 1×10^4 cells/well in triplicate, in a 96-well plate, incubated at 37°C in an oven with 5% CO_2. After 48 h of incubation, the antiviral activity was evaluated through the cytopathic effect and cell viability by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric assay to assess cellular viability. The results are expressed in percentage of viral inactivation (Table 1) through cell viability compared to cell controls in the presence or absence of the virus.

2.3.2.8 Statistical analysis

One-way analysis of variance (ANOVA one-way) and Tukey's and Dunn's tests were applied to statistically evaluate the significant differences between the properties of the samples measured, using the GraphPad Prism 7.04 and a 95% confidence level.

3 Results and discussion

3.1 AgNP suspension

3.1.1 Chemical composition

The AgNP liquid suspension has C (Kα = 0.277 keV) and O (Kα = 0.525 keV) predominantly in its composition, and a trace concentration level of Ag (Lα = 2.984 keV) was identified in the EDS spectrum (Supplementary Figure S1). Sodium (Kα = 1.041 keV) also appears in the AgNP suspension. Amadio and collaborators (Amadio et al., 2018) also identified sodium in this commercial AgNP suspension. According to the results of ICP-OES, the silver and zinc contents in the AgNP suspension are 130 ± 13 mg and 0.02 ± 0.01 mg per milliliter of AgNP suspension, respectively. Copper, another chemical element in the composition of antimicrobial agents commonly used as additives in polymers, was not identified in the antimicrobial suspension by ICP-OES.

The UV-Vis absorption band (Figure 1A) in the 350–500 nm range (absorption maximum at 430 nm) is due to the AgNP surface plasmon resonance (Rehan et al., 2015; Eya'ane Meva et al., 2016). According to the literature (Poisson, 2021), this AgNP suspension is composed not just of silver (Ag) but also of ethylene glycol ($C_2H_6O_2$), poly (vinyl pyrrolidone) (PVP), and water. Ethylene glycol and PVP act as stabilizing agents for AgNPs through a surface-coating stabilization process (Safo et al., 2019).

The FTIR spectrum of the AgNP suspension in Figure 1B presents infrared absorption bands at 860, 885, 1035, 1077, 1215, 1370, 1655, 1733, 2850, 2925, and 3,350 cm^{-1}. The absorption signal at 885 cm^{-1} is related to CH_2 wagging vibrations, and at 1215 cm^{-1} is due to the elongation of C-C bonds (aliphatic carbon) from aliphatic moieties in ethylene glycol (Saikia et al., 2017; Guo et al., 2018). The signal at 3,330 cm^{-1} may be associated with -OH groups from ethylene glycol and water. The absorption bands at 1279 cm^{-1} are related to the vibration of C-N groups on the PVP polymer chains (Safo et al., 2019). The FTIR signal at 1733 cm^{-1} indicates the presence of C=O groups of the ketone group in the pyrrolidone ring of the PVP polymer chains. The signal at 1655 cm^{-1} can be attributed to the vibrations of -OH groups and, also, to the C=O stretching from PVP. This FTIR signal is shifted due to the presence of ethylene glycol and silver in the suspension (Safo et al., 2019). The infrared signals in the FTIR spectrum located at 2925 and 2850 cm^{-1}

TABLE 1 Nomenclature for the antiviral activity[a] assays.

Log reduction	Reduction factor	Inactivation percentage (%)	Activity
1	10	90	Not virucidal
2	100	99	Not virucidal
3	1,000	99.9	Not virucidal
4	10,000	99.99	Virucidal
5	100,000	99.999	Virucidal
6	1,000,000	99.9999	Virucidal

[a]Antiviral activity: difference in the logarithm of virus infectivity titer found in an antiviral-treated product and an untreated product after inoculation and contact with the virus.

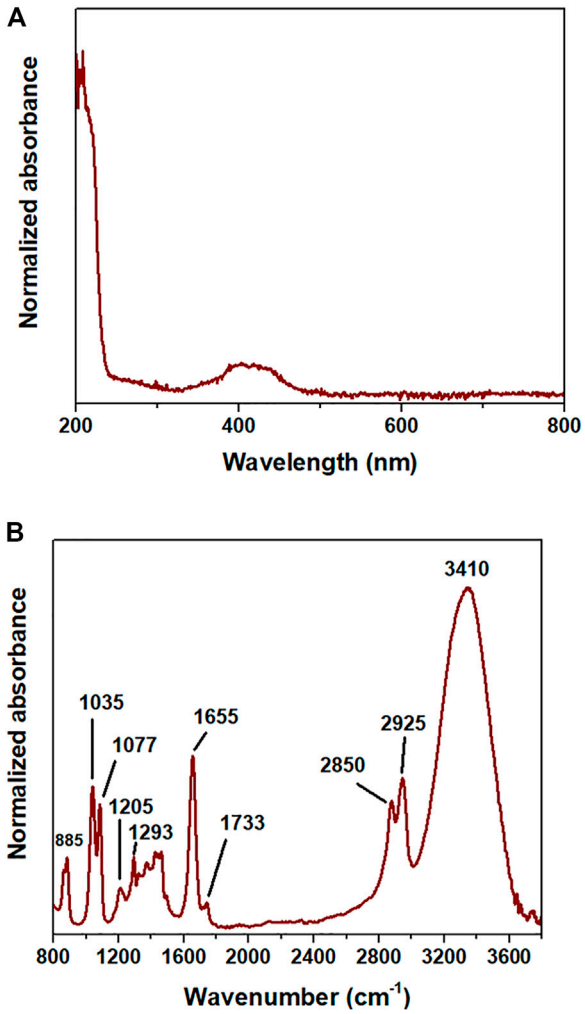

FIGURE 1
(A) UV-Vis spectrum and **(B)** FTIR spectrum of the AgNP suspension.

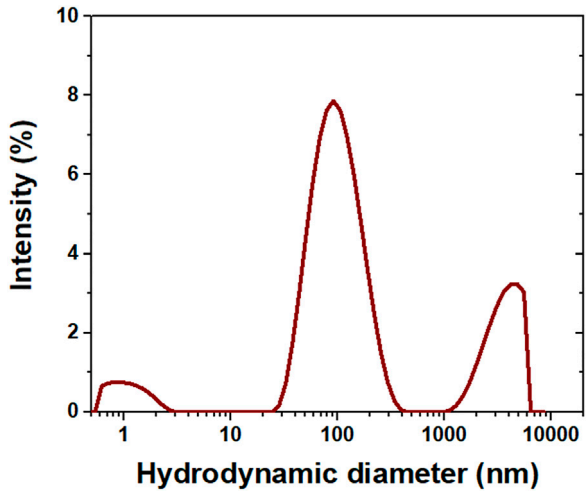

FIGURE 2
Hydrodynamic diameter distribution of the AgNP suspension.

are associated with vibrations of CH groups by asymmetric and symmetric stretching (PVP and ethylene glycol), respectively. Infrared absorption signals at 552 cm^{-1} due to stretching of Ag-O groups of AgNPs stabilized with PVP or ethylene glycol were not detected because it is outside the range of the spectrum analyzed by the ATR-FTIR equipment (Assis et al., 2021).

3.1.2 AgNP particle size

As identified in Figure 2, the suspension presents particles with a trimodal hydrodynamic diameter distribution: less than 10 nm; between 50 and 500 nm; and greater than 1.1 μm ζ value for the AgNP suspension equals −4.7 ± 13.2 mV, indicating that the microparticles detected by DLS are associated with the aggregation of AgNPs in the suspension, which is visually yellowish and transparent. The agglomeration occurs because AgNPs have low electrostatic charges at their surfaces that are insufficient to effectively promote the repulsion between nanoparticles (Shebi and Lisa, 2019). The yellow coloration is similar to the coloration of AgNP suspensions synthesized by different methods reported in different studies in the literature (Rodríguez-León et al., 2013; Kavuličová et al., 2018).

3.2 PVC/AgNP nanocomposites

3.2.1 Scanning electron microscopy (SEM)

Figure 3 presents SEM images for PVC and the PVC/AgNP nanocomposites. According to the supplier, the PVC presents well-dispersed microparticles of calcium carbonate ($CaCO_3$) and

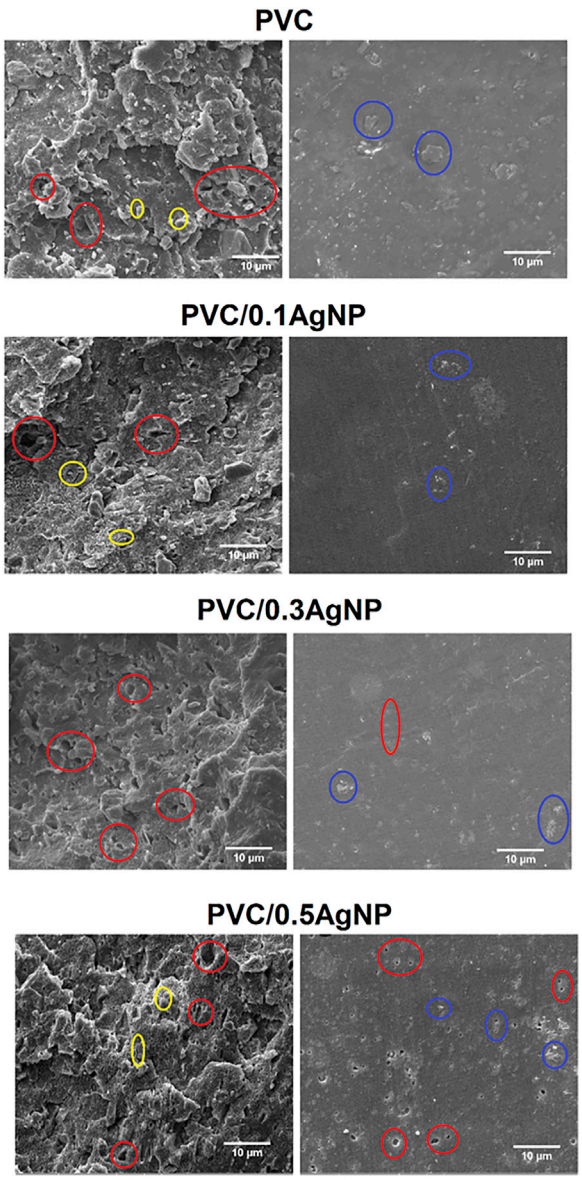

FIGURE 3
SEM images of the PVC and PVC/XAgNP nanocomposites, where X corresponds to the AgNP content (wt%). Images were obtained from the cryofractured internal surfaces (left) and external surfaces (right) of the test specimens. Different defects are highlighted in circles: cavities (red), particles at the exterior surface (blue), and interfacial voids (yellow).

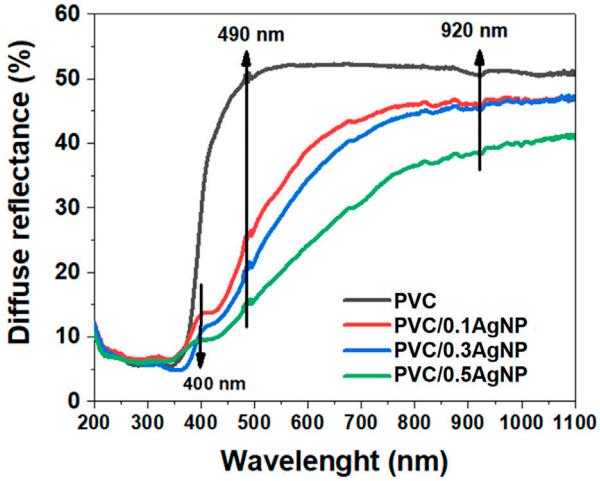

FIGURE 4
Diffuse reflectance (R_d) spectra of the PVC and PVC/XAgNP nanocomposites (X corresponds to the AgNP content in wt%).

titanium dioxide (TiO_2). $CaCO_3$ is an inorganic material widely applied in the polymer industry as a filler to reduce the cost of products based on commodity thermoplastics (Rocha et al., 2018; da Silva et al., 2021a). TiO_2 is extensively utilized in the polymer industry as a white pigment and UV-blocking additive to hamper polymer degradation occasioned by UV exposition (da Silva et al., 2018). Still, TiO_2 also displays photocatalytic properties suitable for self-cleaning coatings on several materials. The usage of solid particles also contributes to diminishing the plasticizer diffusion and migration to the PVC surface and external environment, which leads to substantial changes in the mechanical performance of PVC, in the case of plasticized products (Xiong et al., 2016). Microcavities and interfacial voids are identified at the cryofractured internal surface of the PVC sample due to low adhesion between the filler and polymeric matrix. Moreover, $CaCO_3$ microparticles are visible at the external surface of PVC, which can cause excessive surface roughness of the PVC parts (Figure 3).

The addition of AgNP suspension to the PVC leads to the formation of several microvoids at the external surface, as can be seen on the SEM images of the PVC/0.3AgNP and PVC/0.5AgNP nanocomposites (Figure 3). It occurs due to the evaporation of volatile compounds in the AgNP suspension, which is caused by heating during the thermal processing of the PVC samples. AgNP suspension also seems to increase the number of microvoids at the cryofractured internal surface of the PVC/1AgNP nanocomposite, suggesting a more significant detachment of the $CaCO_3$ particles from the PVC matrix that is justified by the poor interfacial adhesion between the phases (da Silva et al., 2018). Even knowing the size of the AgNP and AgNP aggregates in the antimicrobial silver suspension by DLS, it is impossible to identify them in the SEM images due to the low concentration of AgNP.

The SEM images in Figure 3 also show that the fracture of the PVC matrix changes from brittle to ductile due to the increase in AgNP content. This result may be associated with the small organic molecules in this silver suspension that increase the polymer chain mobility in the composite even with the presence of a micrometric filler, acting as a plasticizing agent and enabling more plastic deformation in the PVC matrix.

3.2.2 UV-vis diffusive reflectance spectroscopy

The diffuse reflectance (R_d) spectra of the PVC and the nanocomposite samples are shown in Figure 4. PVC and all nanocomposites present an anomalous light dispersion at 490 nm due to an abrupt and concomitant increase in the absorptivity and refractive index of the PVC system. This phenomenon is called the Eststrahlen effect, which is associated with a predominant Fresnel reflectance over the Kubeika-Munk reflectance at this specific

TABLE 2 The visual aspect, yellowness index (YI), direct (E_g^d) and indirect (E_g^i) optical bandgaps of the PVC and PVC/XAgNP nanocomposites (X corresponds to the AgNP content in wt%).

Sample	Visual aspect	YI (%)	E_g^d (eV)	E_g^i (eV)
PVC		4.4	3.1	3.0
PVC/0.1AgNP		16.8	2.6	2.2
PVC/0.3AgNP		23.0	2.5	1.8
PVC/0.5AgNP		28.6	2.5	1.5

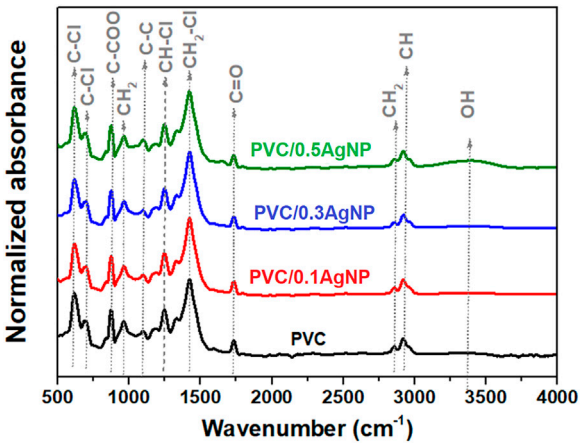

FIGURE 5
FTIR spectra from the PVC and PVC/XAgNP nanocomposites (X corresponds to the AgNP content in wt%).

wavelength (Blitz, 1998; Tao et al., 2015). The PVC system displays an absorption signal at 920 nm in the near-infrared (NIR) wavelength region, in which its signal intensity is reduced as the AgNP content rises in PVC. The reason for this spectral phenomenon at 920 nm is out of our awareness, but it can be connected to the lamp change at 350 nm of the UV-Vis equipment (from deuterium to halogen lamp) during the measurements. The R_d intensity also decreases with the increase of the AgNP concentration in the PVC.

It is reported that PVC exhibits absorption maxima at 280 and 245 nm in the UV region due to π–π* electronic transitions in the polymer backbones (Abdel-Fattah et al., 2019). However, the PVC used in this work presents a sharp absorption profile after 450 nm, and the R_d intensities are minimal at 350 nm. This UV-visible absorption profile is similar to $CaCO_3/TiO_2$ hybrid particles, which are utilized commercially as an alternative white pigment to work around the higher price of TiO_2 pigment (more expensive than $CaCO_3$) and the scarcity of titanium resources (Sun et al., 2018).

According to Figure 4, the addition of AgNPs in the PVC leads to a broadening of the R_d intensity for higher wavelengths (redshift) occasioned by an enhancement of the absorption coefficient in the visible wavelength. The low bandgaps of the inorganic components in the PVC/AgNP nanocomposites are responsible for these results since they have optical bandgaps (E_g) inferior to that of PVC. It is well known that silver and other noble metals reduce the optical bandgap of semiconducting metallic oxides, improving their UV-visible light absorption due to the introduction of lower energetic levels in the electron energy band structure of the semiconductor (Abbad et al., 2020). Moreover, the absorption of visible radiation by the AgNP plasmon resonance states with low-energetic levels must contribute to the absorption broadening in the UV-visible electromagnetic region, including the reduction of the direct (E_g^d) and indirect (E_g^i) optical bandgaps of the PVC and PVC/AgNP nanocomposites obtained from Tauc's plot and Kubelka-Munk transformation here (Supplementary Figure 2S).

E_g^d and E_g^i values are detailed in Table 2. The optical bandgaps are close to those experimentally observed for TiO_2 that displays bandgap around 3.2 and 2.9 eV for anatase and rutile phases, respectively (Ivanova et al., 2016; Munir et al., 2019). The anatase phase has an indirect bandgap, while the rutile presents direct electronic transitions (Ramos Jr et al., 2017). Abdel-Fattah et al. (2019) reported the direct and indirect optical bandgaps of PVC film around 4.2–4.3 eV, which are values higher than those experimentally observed in our PVC system. According to Ghadam et al. (Ghadam and Idrees, 2013), calcite ($CaCO_3$) is an indirect bandgap semiconductor with E_g^i very close to 5.8 eV. Then, all this information from the literature indicates that the bandgap data of the PVC in Table 2 are relative to TiO_2. Also, increasing AgNP in the PVC nanocomposites slightly reduces the E_g^d and E_g^i of this oxide, as expected and explained previously (Abbad et al., 2020; Gogoi et al., 2020). Antagonistically, the yellowness index (YI) of the PVC enhances as the AgNP content increases in the PVC/AgNP nanocomposites due to the characteristic yellow color of the AgNP suspension. The YI values calculated here are coherent with the yellowish coloring aspect of the PVC samples visually observable in Table 2.

3.2.3 Fourier-transform infrared absorption spectroscopy (FTIR)

Figure 5 presents the FTIR spectra of the PVC samples. There are infrared absorption signals associated with molecular vibrations of distinct chemical functional groups from PVC (Coltro et al., 2013; Park et al., 2018): C-Cl (stretching, 610 and 695 cm^{-1}), CH_2 (asymmetrical stretching, 2851 cm^{-1}), C-C (stretching, 1100 cm^{-1}), CH-Cl (out-of-plane angular deformation, 1253 cm^{-1}), CH_2–Cl (angular deformation, 1425 cm^{-1}).

The FTIR signal at 870 cm^{-1} is associated with the C-COO bond, confirming the presence of calcium carbonate ($CaCO_3$) as indicated by SEM images. Moreover, there are infrared absorption signals from carbonyl (1722 cm^{-1}) and polyene (1602 cm^{-1}) groups due to PVC thermooxidative degradation (Yousif et al., 2016). PVC degrades mainly by dehydrochlorination, releasing HCl with the generation of polyenes. However, chloroketones and aliphatic ketones also can be formed by alternative degradation reaction mechanisms of this polymer with oxygen gas in the atmosphere (Yousif et al., 2016; Yu et al., 2016). Also, the polyenes can suffer

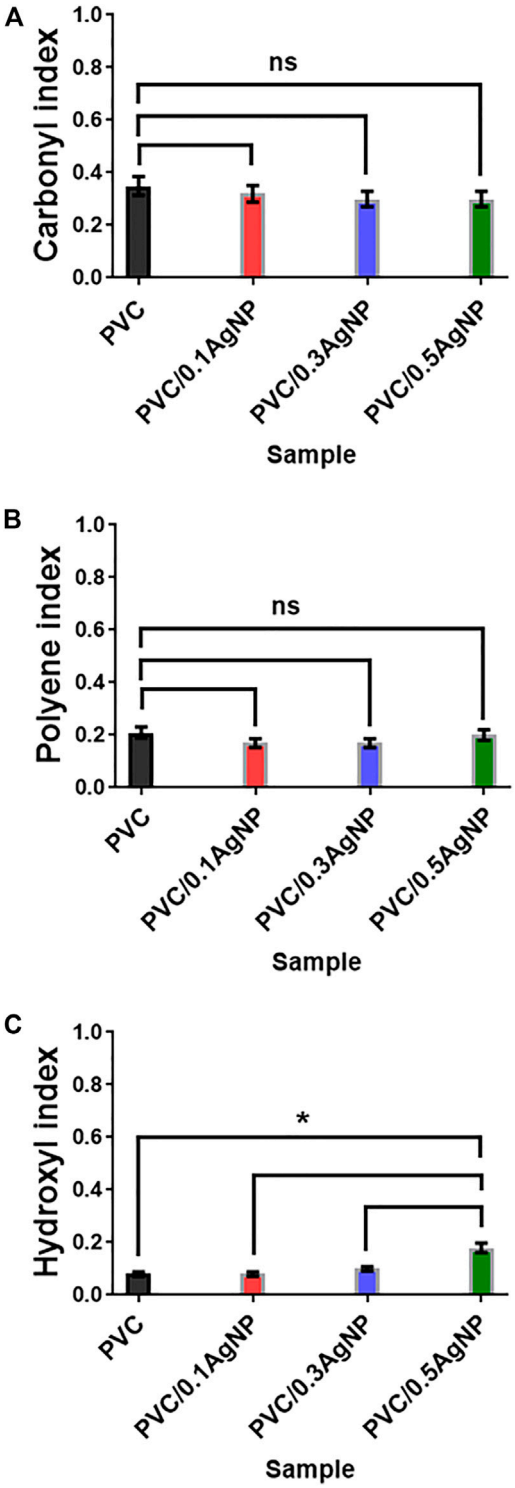

FIGURE 6
(A) Carbonyl ($I_{C=O}$), **(B)** polyene ($I_{C=C}$), and **(C)** hydroxyl (I_{OH}) indexes from FTIR spectra of the PVC and PVC/XAgNP nanocomposites (X corresponds to the AgNP content). Statistical analyses are for each sample group, using Tukey's multiple comparison tests. ns = data are not significantly different (*p*-value >0.05).

crosslinking reactions *via* Diels–Alder condensation, generating C=C bonds in cyclic compounds (Morikawa, 2014). As shown in Figures 6A, B, the carbonyl ($I_{C=O}$) and polyene ($I_{C=C}$) indexes have no significant differences, indicating that the addition of the AgNP suspension did not intensify the PVC degradation during thermomechanical mixing.

PVC photooxidation reactions due to UV irradiation lead to polymer chain scissions with increasing hydroxyl groups in the polymer (3,500 cm^{-1}) (Yousif and Hasan, 2015). However, the hydroxyl index (I_{OH}) from the PVC (Figure 6C) was significantly enhanced by adding 0.5 wt% of AgNP, which is expected by the presence of OH groups from components in the silver suspension (PVP and ethylene glycol).

3.2.4 X-ray photoelectron spectroscopy (XPS)

The XPS spectra of binding energies for carbon bonds (C1s XPS region) in PVC samples are shown in Figure 7. Four C1s fitting peaks are identified in PVC at 284.8 eV (C–C/C–H), 286.3 eV (C–Cl), 284 eV (C=C), and 288.1 eV (C-O) (Wang et al., 2015; Fu et al., 2019; Baibarac et al., 2021). The PVC nanocomposites present another XPS peak at 287.5 eV from C-N bonds, shifting the XPS signal from C-O bonds to 289–290 eV. The C-N bonds are associated with stabilizer compounds in the AgNP suspension, such as poly (vinyl pyrrolidone) (PVP). The presence of C=C bonds at the surface of the PVC samples corroborates the FTIR and UV-Vis data that indicate PVC degradation.

The Ag3d XPS spectra (Figure 8) confirm the presence of Ag (0) (metallic silver) in the PVC nanocomposites due to the presence of XPS signal peaks at 372.2 ($Ag3d_{3/2}$) and 365.5 eV ($Ag3d_{5/2}$) (Sharma et al., 2018). The low intensity of Ag (0) signal can be associated with the attenuation of electrons caused by the capping effects of the AgNPs by PVP and ethylene glycol (Binaymotlagh et al., 2022).

The XPS depth-profile results in Supplementary Figure S3 indicate that the PVC sample undergoes a more pronounced dehydrochlorination degradative process at the surface than the PVC nanocomposites, since the C-Cl peak area increases while the C=C peak area reduces along the sample depth. The silver suspension seems to ease the localized thermooxidative degradation at the PVC surface due to the local heating during the molding. Moreover, the XPS depth-profile data suggest that silver nanoparticles are distributed within the PVC/AgNP nanocomposites, which is essential to their antimicrobial performance in applications where the surface is subject to constant wear to maintain the AgNP content at the PVC nanocomposites consistently higher than the minimum antimicrobial concentration.

3.2.5 Mechanical properties

Young's modulus (E) and ultimate tensile strength (σ_{max}) from uniaxial tensile tests of the PVC and PVC/AgNP nanocomposites are shown in Figure 9. PVC had a tensile strength of 45.1 ± 4.9 MPa and a tensile modulus of 2.1 ± 0.3 GPa. The PVC and all composites were tested at the same ASTM standard and strain rates, enabling a direct comparison of the uniaxial tensile measurements. For this

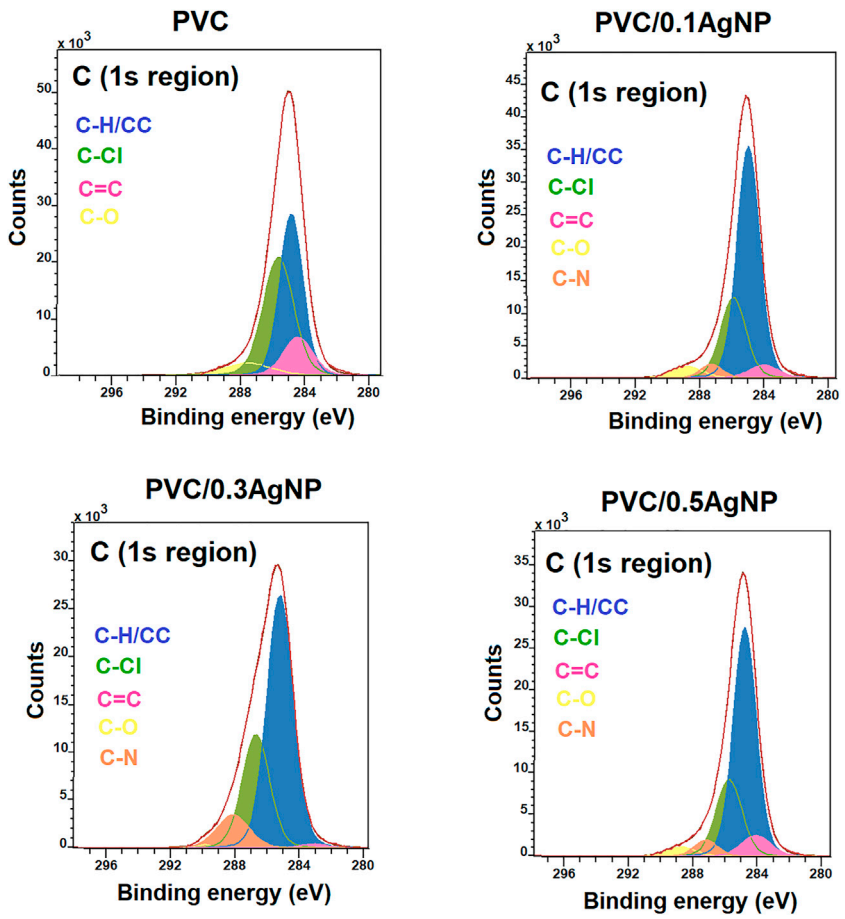

FIGURE 7
C1s XPS high-resolution spectra from the PVC and PVC/*X*AgNP nanocomposites (*X* corresponds to the AgNP content in wt%).

purpose, we applied Tukey's multiple comparison test as ANOVA one-way method where the results are considered significantly different if the *p*-value is lower than 0.05 using a 95% confidence level. The PVC nanocomposites present Young's moduli equal to 1.7 ± 0.1, 1.6 ± 0.1, and 1.4 ± 0.1 GPa when added 0.1, 0.3, and 0.5 wt % of AgNP in the PVC, respectively. According to ANOVA, Young's moduli of the PVC/AgNP nanocomposites are identical. However, they are significantly lower than the E value of the PVC, probably due to the local plasticizing effect of the AgNP suspension on the PVC observed in the SEM images *via* lubricant or gel swelling mechanisms (Daniels, 2009; Quesada-Pérez et al., 2011; Langlois and Deville, 2014). Consequently, the immobilization of the polymer matrix due to inorganic particle stiffness does not contribute significantly to the enhancement of Young's modulus of the PVC (Watt et al., 2020).

The ultimate tensile strengths of the PVC/AgNP nanocomposites are also statistically equal, independently of the AgNP content, as shown in Figure 9B. However, the σ_{max} data from the PVC is slightly superior to those from PVC/0.3AgNP and PVC/0.5AgNP nanocomposites (around 32–41 MPa), confirming the hypothesis that the AgNP suspension reduces the adhesion between the PVC matrix with the inorganic microparticles as suggested by SEM analysis. This reduction in the interfacial adhesion leads to a poor stress transfer between these phases, causing a decrease of σ_{max} for the composite.

The toughness of the PVC samples was evaluated by Izod impact tests, and the results are shown in Figure 10. There is no significant difference in impact strength for PVC with the increase of the AgNP concentration, despite the increase of cavities on the PVC surface caused by the insertion of AgNPs. This result is important, as the antimicrobial grade PVC must have similar toughness to the original commercial PVC compounds used to produce parts for hospital environments. The impact strength results are 100 ± 9 (PVC), 103 ± 14 (PVC/0.1AgNP), 99 ± 16 (PVC/0.3AgNP), and 113 ± 24 J m^{-1} (PVC/0.5AgNP). In another way, the decrease in strength and toughness of PVC/AgNP nanocomposites due to the presence of microstructural defects caused by AgNPs was reported by Merchan et al. (2010). Braga et al. (2019) observed a similar reduction of mechanical strength of the PVC films (prepared by solvent casting method) caused by AgNP aggregation. According to them, the silver nanoparticles at concentrations of 2 – 8 wt% generated a less cohesive internal structure, affecting mechanical strength and also decreasing the elongation at the break of the PVC (Braga et al., 2019).

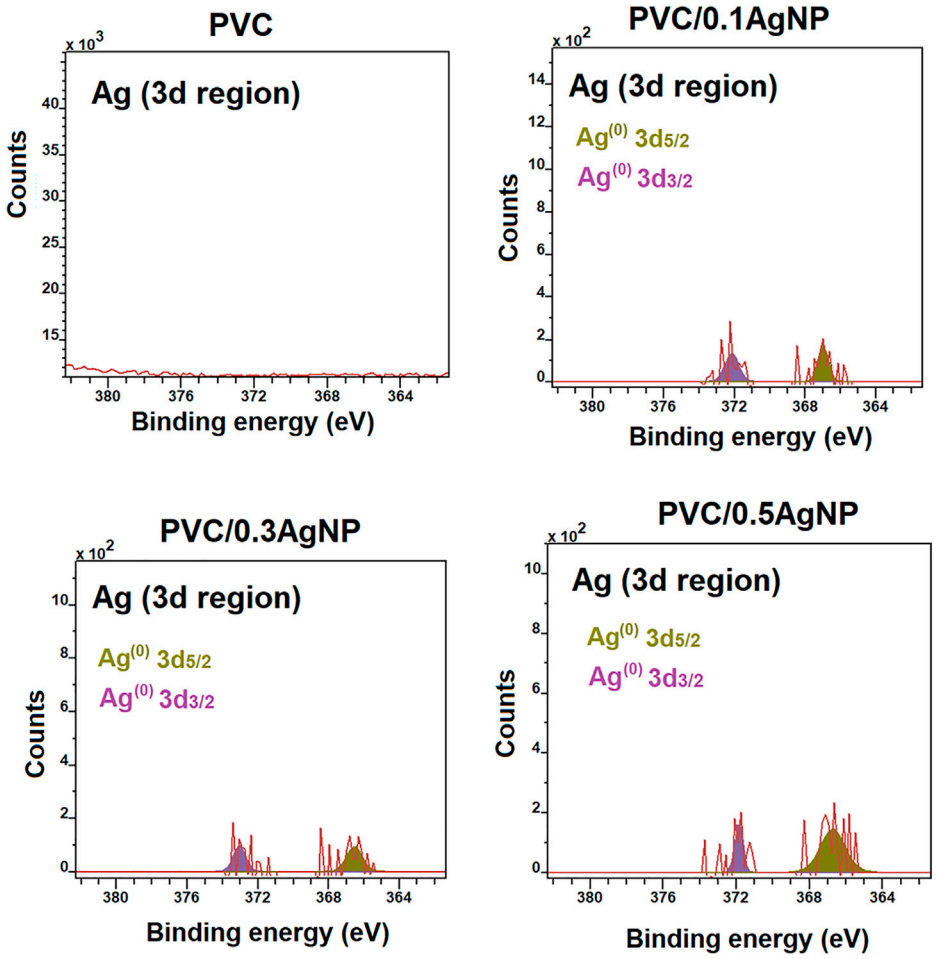

FIGURE 8
Ag3d XPS high-resolution spectra from the PVC and PVC/*X*AgNP nanocomposites (*X* corresponds to the AgNP concentration).

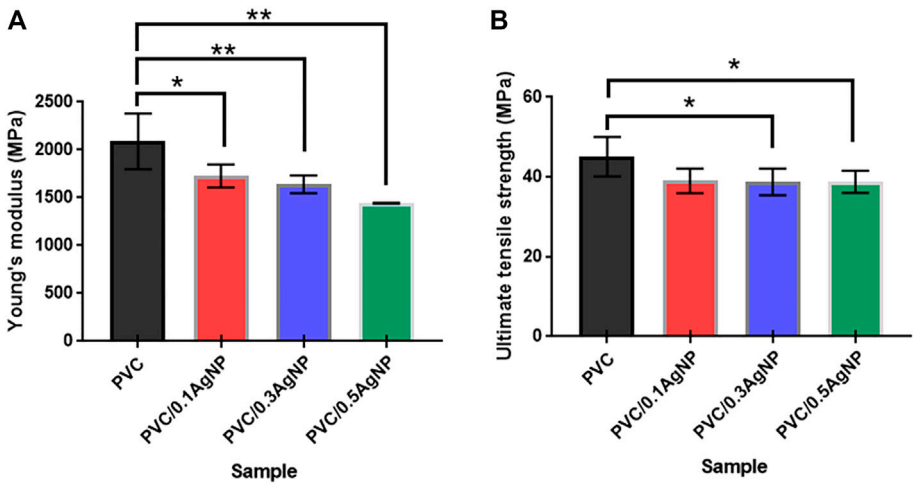

FIGURE 9
(A) Young's modulus and **(B)** ultimate tensile strength of the PVC and PVC/XAgNP nanocomposites, where X corresponds to the AgNP content. The data represent mean ± standard deviation (SD) (n = 3–6). Statistical analyses are for each sample group, using Tukey's multiple comparison tests. The *p*-value is considered significant at <0.05 (95% confidence level). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ indicate mean data significantly different.

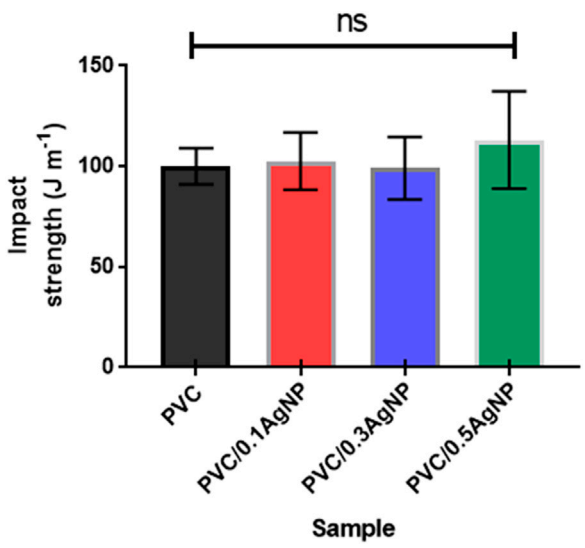

FIGURE 10
Izod impact strength of the PVC and PVC/XAgNP nanocomposites, where X corresponds to the AgNP content (wt%). The data represent mean ± SD (n = 5). Statistical analyses are for each sample group, using Tukey's multiple comparison tests. ns = data are not significantly different (p-value >0.05).

3.2.6 Thermogravimetric analysis (TGA)

Figure 11 presents the thermal decomposition profiles of the polymeric samples. PVC thermally decomposes *via* two distinct stages. From 250°C to 350 °C, the major mass loss (50 wt%) occurs due to the PVC dehydrochlorination with the formation of polyene sequences along the PVC polymer backbone (Kayyarapu et al., 2016). The second stage, from 420°C to 550 °C, involves the mass loss of around 20 wt% associated with the decomposition of the polyene sequences, generating carbonaceous residues (Cruz et al., 2021) that remain along with the inorganic particles (identified by SEM).

All PVC samples have similar onset thermal decomposition temperatures (T_{onset}), varying from 276°C to 289 °C, as detailed in Table 3. From the DTG curves, the temperatures at the maximum thermal decomposition rate (T_{max}) of each stage were determined. There is a slight rising on the T_{max} average values from the PVC's first thermal decomposition step due to the increase in the AgNP content. The silver nanoparticles may hamper the loss of volatiles generated by the PVC dehydrochlorination reactions during its heating. T_{max} is associated with the PVC second thermal decomposition step and is not affected by the AgNP content. Shimoga et al. (2019) observed the opposite effect of AgNP concentration on T_{onset} for the first thermal decomposition step in AgNP/PVC films obtained by casting. They attributed the low thermal stability of AgNP/PVC films to the solvent molecules trapped between the polymer chains that caused thermal decomposition of PVC at temperatures below 200°C (Shimoga et al., 2019). Furthermore, adding 0.5 wt% of AgNPs provided the highest total weight loss for the nanocomposites at 600°C, which is justified by the higher amount of low-mass organic compounds in the AgNP suspension that are thermally decomposed in the PVC matrix above 400 °C. Braga et al. (2019) also observed that the dehydrochlorination onset temperature of AgNP/PVC films decreases with the enhancement of AgNP concentration, but it causes a reduction of the total weight loss since the content of inorganic materials increases in the PVC matrix.

TABLE 3 T_{onset} **and** T_{max} **temperatures from TGA and DTG measurements of the PVC samples.**

Sample	T_{onset} (°C)	T_{max} (°C)
PVC	281 ± 5	302 ± 75
		460 ± 78
PVC/0.1AgNP	282 ± 5	307 ± 75
		460 ± 78
PVC/0.3AgNP	283 ± 5	311 ± 75
		460 ± 78
PVC/0.5AgNP	284 ± 5	311 ± 75
		460 ± 78

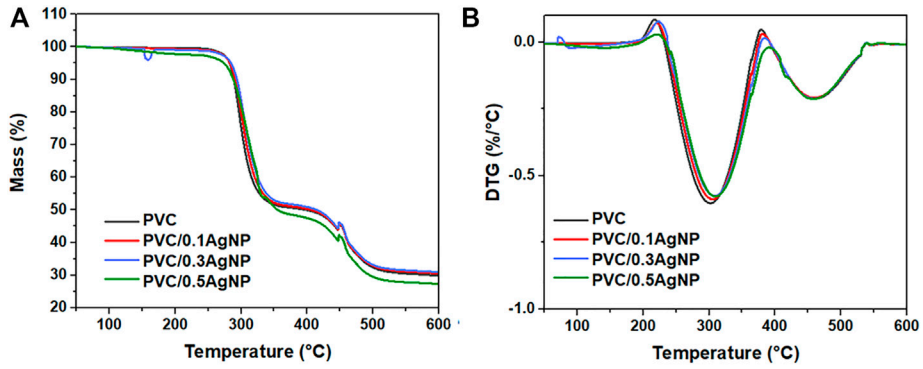

FIGURE 11
TGA thermograms **(A)** and the DTG curves **(B)** from the PVC and PVC/XAgNP nanocomposites, where X corresponds to the AgNP content (wt%).

TABLE 4 Antiviral activity results from the PVC and PVC/XAgNP nanocomposites.

Sample	Log reduction	Inactivation percentage (%)	Activity
PVC	1	90	Not virucidal
PVC/0.1AgNP	2	99	Not virucidal
PVC/0.3AgNP	4	99.99	Virucidal
PVC/0.5AgNP	4	99.99	Virucidal

[a]Results are expressed as a viral inactivation percentage through cell viability compared to cell controls in the presence or absence of SARS-CoV-2.

3.2.7 Antiviral assays

The assay was performed at different contact times, with 30, 60, and 120 min (Supplementary Figure S4a). The percentage of viral inactivation observed through cell viability increases with longer contact times. PVC/0.3AgNP and PVC/0.5AgNP samples present virucidal activity compared to cell controls with significant differences against the SARS-CoV-2 positive control, according to Dunn's tests (Table 4; Supplementary Figure S4a). Therefore, to achieve an inactivation percentage of 99.99% in 48 h, the PVC must contain at least 0.3 wt% of AgNPs. The antiviral activities from these nanocomposites were evidenced by the decrease in the cytopathic effects caused by the virus that reduced the percentage of viable cells. There are few published works on the antiviral activity of polymer matrix nanocomposites against SARS-CoV-2 variants. Lam et al. (2022) reported that polyurethane/AgNP nanocomposites could reduce the amount of SARS-CoV-2 beta (B.1.351) virions by 67% within 24 h of direct contact antiviral assays. According to $TCID_{50}$ reduction assays, Assis et al. (2022) observed that SARS-CoV-2 antiviral activity of propylene (PP) composites with 0.3 wt% of Ag_2XO_4 (X = W, Mo, and Cr).

The time-dependent virucidal effect of the samples is directly associated with increased contact time due to the longer exposure time of virions to AgNPs, Ag^+ ions, and ROS that cause irreversible damage to viral particles (da Silva et al., 2021b). Jeremiah et al. (2020) also observed time-dependent virucidal effects of AgNP suspensions against SARS-CoV-2 virions.

4 Conclusion

In this work, PVC/AgNP nanocomposites were successfully prepared *via* melt mixing, which is a suitable route for the large-scale production of polymeric products with large sizes and complex geometries. SEM images evidence the formation of surface defects on the PVC due to the addition of AgNPs, leading to changes in Young's modulus and ultimate tensile mainly when the AgNP content is higher than 0.1 wt%. The toughness of the PVC/AgNP nanocomposites is similar to the PVC. TGA and FTIR data indicate that the AgNPs do not lead to significant degradation of the PVC matrix bulk. According to the XPS high-resolution depth-profile measurements, the AgNP suspension prevented located dehydrochlorination degradation of the PVC matrix at the surface of the PVC/AgNP nanocomposites.

UV-Vis spectroscopy evidences an increase in the PVC's yellowness index (YI) due to the increased AgNP content, causing visual changes inherent to the compounds with yellow color in the AgNP suspension. The cytopathic effect and cell viability assays proved that the nanocomposites present virucidal activity against SARS-CoV-2 within 48 min if the AgNP content is at least 0.3 wt%. The antiviral nanocomposites seem adequate for application on plastic objects to reduce the transmission of COVID-19, mainly in environments with high biological risks of exposure to transmitting viral diseases through contact with contaminated surfaces, such as hospitals and medical clinics.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

DS: Conceptualization, methodology, validation, formal analysis, investigation, writing—original draft, writing—review and editing, visualization. GG: Experimental work, methodology, formal analysis, investigation PJ: Experimental work, methodology, formal analysis, investigation. VM: Experimental work, methodology, formal analysis, investigation, validation. AM: Experimental work, methodology. MH: Validation, resources, writing—review and editing, supervision, project administration, funding acquisition. SL: Resources, review, supervision, project administration, funding acquisition. LB: Resources, review, supervision, project administration, funding acquisition. MC: Experimental work, conceptualization, methodology, validation, formal analysis, investigation, review, visualization, supervision, project administration. DC: Experimental work, methodology, validation, formal analysis, investigation, writing—review and editing, visualization, supervision, project administration. All authors approved the manuscript.

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Conflict of interest

SL was employed by BRGoods Indústria e Comércio de Produtos Hospitalares.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that all funders were not involved in the collection, analysis, interpretation of the study design and data, or the writing or submission of the article for publication decisions.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2023.1083399/full#supplementary-material

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The impact of mean body mass index on reported mortality from COVID-19 across 181 countries

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Accountability for global health issues such as a pandemic and its devastating consequences are usually ascribed to a virus, but a comprehensive view should also take into account the state of the host. Data suggests that excessive nutrition is to blame for a yet unknown but not negligible portion of deaths attributed to severe acute respiratory syndrome coronavirus 2. We analyzed the correlation between mean body mass index (BMI) and 2-year coronavirus disease 2019 (COVID-19) mortality rates reported by 181 countries worldwide. Almost two thirds of the countries included had a mean BMI greater or equal to 25, with death rates ranging from 3 to 6,280 per million. Death rates in countries with a mean BMI below 25 ranged from 3 to 1,533. When the analysis was restricted to countries where the extent of testing was deemed more representative of actual mortality, only 20.1% had a mean BMI<25 but the mortality difference persisted. A second analysis looking at pre-vaccination mortality obtained from a different source led to similar conclusions. Due to the nature of the variables, reverse causation can be excluded while common causation can not. A mean BMI<25 for a country seems to spare its citizens from the highest COVID-19 mortality rates. The impact of excess weight on global COVID-19 mortality is suspected to have been much higher than what currently perceived, here estimated at no less than a fourfold increase in mortality. Countries with normal mean BMI constitute precious test beds for the quantification of the effects of overeating on COVID-19 mortality.

KEYWORDS

body mass index, COVID-19, mortality, public health, overeating

Introduction

Koch's third postulate states that in order to establish a causal relationship between a microbe and a disease, the microorganism should cause disease when introduced into a *healthy organism*. No reference is made to the severity of the disease caused, nor to what constitutes a healthy organism, nor to the possibility for the organism to be in a state of partial well-being.

Hill improved upon Koch's original criteria in many aspects, but the difficulties encountered in the quantification of the initial state of health of the host and how this could affect both infectivity and mortality remain. An organism can at any time and for a variety of different reasons weaken a subset of its own defense systems, with no apparent ill effects. It is only when a new threat that specifically exploits that very weakness that the host begins to tumble.

For this reason we asked ourselves whether the COVID-19 pandemic—had the virus not found a species afflicted by overeating—would have been as deadly as it had.

Obesity, once considered by many clinicians a self-inflicted condition of little medical significance, has increased dramatically during the last four decades (1). Today we know that it carries higher risks for the development of type 2 diabetes, coronary heart disease, a number of cancers, respiratory complications and osteoarthritis. Even more modest degrees of overweight are associated with mortality (2). Health care resources are inundated by obesity and its consequences (3), with high social and economic costs, including attempts to prevent or to treat it (4).

Although failure to mention obesity as one of the preexisting diseases associated with death still occurs (5), individuals with obesity, overall and central, are more at risk for being COVID-19 positive [46% more (6)] (7–9), hospitalization (10), ICU admission (6, 9), reinfection (11) and mortality [48% more (6)] (7). Obesity and impaired metabolic health are important risk factors for severe COVID-19 (12–16). The risk of hospital admission or death due to COVID-19 starts at a body mass index (BMI) as low as 23 kg/m^2 (13). Central obesity and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine (17). Hypertension was found to be more prevalent in the first surge of the disease in Iran, where patients were also younger (18). SARS-CoV-2 infection induces neutralizing antibodies only in few obese COVID-19 patients (19). Given that BMI has a causal role in the development of severe COVID-19, the promotion of weight loss in people with obesity or overweight would help to combat the COVID-19 pandemic (12). In addition to increased risks for the subject, people with high BMI were also found to transmit the infection more easily. The number of exhaled respiratory droplets were in fact considerably higher for these individuals, and further increased with degree of COVID-19 infection (20). A positive association with death was found in previously hospitalized individuals for BMI >37 (21) and among infected underweight, obesity class II and III patients (22).

Among the very old, overweight and obesity were found not to be associated with in-hospital mortality (23). For patients aged ≥60 year, mild/moderate obesity was associated with a 13% reduced mortality risk and a 10% increased length of stay in the ICU (24). Although underweight patients had a higher risk of mortality from COVID-19 (25), overweight and obese patients outnumber them in most countries, making an analysis of mean values not futile.

The combination of increased infection rates, increased transmissibility and decreased antibody response to vaccine, all lead to a larger basic reproduction number. Quantification of the mutual amplification of such effects remains a challenge. Since the majority of the population in many high-income countries is overweight, and since both overweight adolescents and adults experience more respiratory symptoms (26), the role played by excess weight on the intensity of the initial viral diffusion and on its temporal and spatial evolution across the globe should not be ignored.

At this time, doubt pushed us to explore the patterns of mortality around the globe, and their relation to BMI, trying to go beyond both the limitations of BMI itself (definitions of overweight range from BMI ≥23 to BMI ≥25) and our struggle with estimations of exact death tolls (27). In the era of globalization, a survey of this kind might not come as handy as one might expect, but if interpreted correctly it is still revealing.

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Materials and methods

The first problem we encountered was to assess data comparability. Since under-testing and under-reporting of deaths is believed to be common in developing countries, we initially considered analyzing the correlation between COVID-19 tests per capita and reported COVID-19 deaths. The analyses included over 180 countries for which both BMI and mortality data were present. We then conducted very basic observations on the patterns of correlation between COVID-19 mortality and mean BMI, plus three additional variables that for different reasons we felt could potentially have an influence: cigarette consumption [chronic obstructive pulmonary disease worsens outcomes from COVID-19 (28)], annual average precipitation and average temperature (29, 30). Additional indices were looked at, such as the gross domestic product at purchasing power parity, but it was felt that its impact on mortality, although significant, required a socioeconomic type of analysis, which was beyond the scope of this work.

Data for each country was initially collected from a number of different sources and collated into a single file. This included mean BMI from the World Health Organization's Global Status Report on noncommunicable diseases (WHO) (31), population, COVID-19 deaths and tests from the global statistics provider Worldometers (32) and the project Our World in Data (33) (OWID), life expectancy at birth in 2019 from WHO, annual cigarette consumption per person from the Tobacco Atlas (34), annual average precipitation from the World Bank (35) and average temperature from the Climatic Research Unit (36). Data extracted from OWID, in addition to deaths and tests, included the day COVID-19 statistics recording began. This was functional to derive annual quantities, considering that different countries started official recording at different times.

A first correlation between BMI and COVID-19 deaths was evidenced by data retrieved from WHO and the statistics provider Worldometer. Countries with limited testing also reported few deaths. To limit the risk of introducing less reliable data, we restricted our analysis to countries who tested beyond a specific value. This value was chosen based on the distribution of the data itself. No sharp increase in mortality was present for an increment in testing rate up to about 0.1 tests per capita (50 tests per thousand people per year). The analysis was therefore split into two groups: one containing countries who tested up to 0.1 per capita (41) and the other containing countries who tested more (134). Six countries did not have data on the number of administered tests and were excluded (Figure 1A).

In order to eliminate the effect of vaccination, and also as a mean of comparison and validation against the first database, a second COVID-19 database was used. This was freely available from OWID. For each country, the day data were first recorded was extracted. This start date was used to compute the interval of time across which mortality was reported. Total COVID deaths, tests and population of each country were also extracted. The analysis was restricted to year 2020. Using a combination of basic command

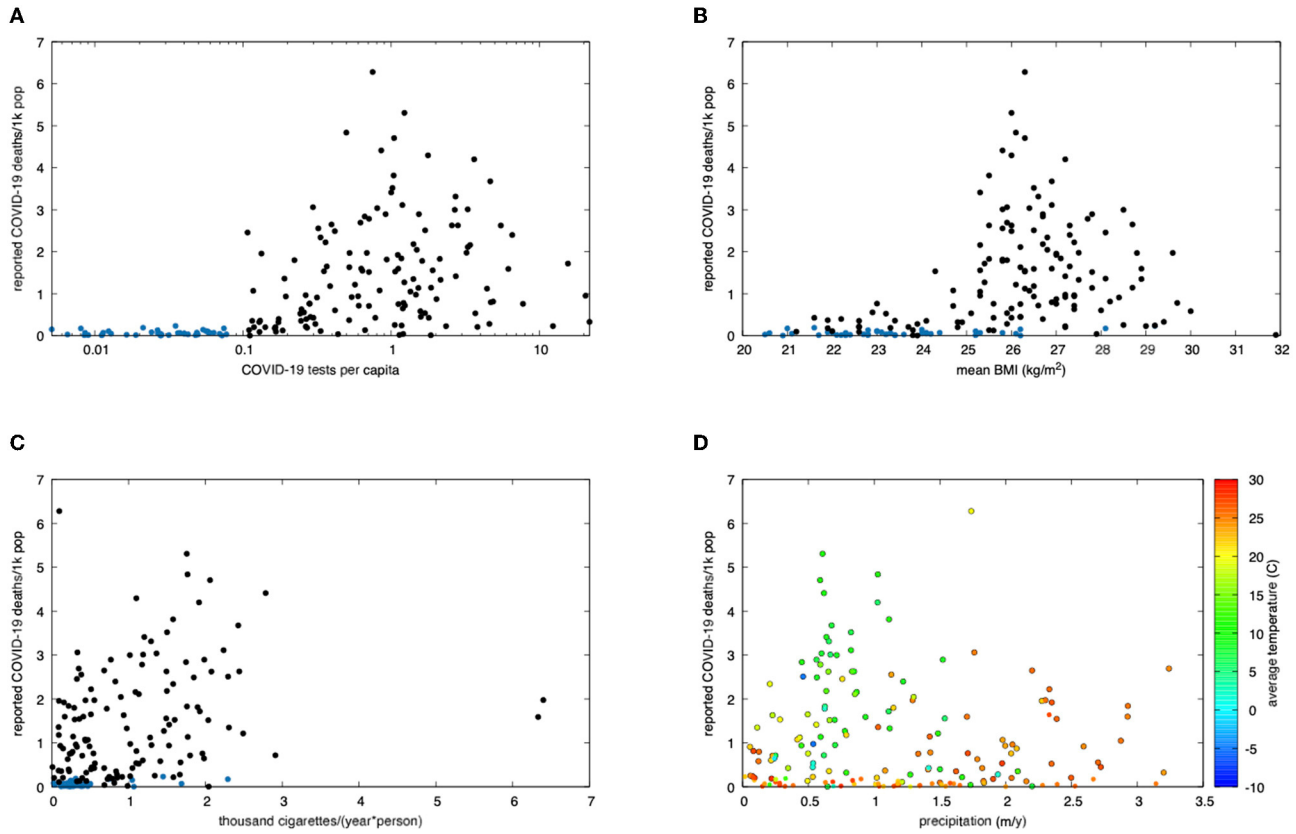

FIGURE 1
Correlation plots analysing COVID-19 mortality as a function of BMI, cigarette consumption, annual precipitations and average temperature in 181 countries worldwide COVID-19 data covers about 2 years, from initial counting until 25 March 2022. Purportedly under-reporting countries are shown in blue in the first three panels and edgeless in the last one. **(A)** Scattered plot of reported COVID-19 deaths vs. tests per capita (worldometers.info, retrieved on 25 March 2022). Under-reporting of deaths seems more likely below about 50 tests per thousand people per year. **(B)** Scattered plot of reported deaths per thousand due to COVID-19 (worldometers.info) vs. mean BMI (31). **(C)** Scattered plot of reported deaths per thousand due to COVID-19 vs. cigarette smoking (tobaccoatlas.org, 2016). **(D)** Average annual precipitation (35) and temperature (1961–1990 data, Climatic Research Unit, 2011). Warmer and humid areas experienced reduced mortality. Data source: Mitchell, T.D., Carter, T.R., Jones, P.D., Hulme, M., New, M., 2003: A Comprehensive Set of High-Resolution Grids of Monthly Climate for Europe and the Globe: the Observed Record (1901–2000) and 16 Scenarios (2001–2100). J. Climate: submitted.

line tools and a spreadsheet, a data file was generated. Three more columns were added: one for death rates, one for test rates and one for BMI. The World Health Organization's Global Status Report on noncommunicable diseases 2014 contains both 2010 and 2014 BMI data, which show increases of up to 2% over a period of 4 years, hovering around 1% for most countries. Given that COVID-19 struck 6 years later, and that adult BMI has and is seeing a linear increase worldwide (37), our analysis underestimates the effect of BMI on mortality. Regarding causality, reverse causation is for temporality reasons not possible. Common causative factors—if any is present—would call for a proper quantification of their individual impact.

Out of 214 countries, 31 had no BMI data, thus allowing analysis of 183 countries in total. All data files are available as Supplemental material.

Results

Plotting each country's COVID-19 death rate vs. its mean BMI gave us some elementary clues (Figure 1B). After over 2 years into the pandemic, countries with a BMI<25 reached a maximum of 1,535 deaths (Namibia), as opposed to those exceeding 25, where death rates soared, up to 6,280 (Peru, see Supplemental material). Correlation with cigarette smoking was much weaker, as shown in Figure 1C. The effect of climate is shown in Figure 1D, where both precipitations and higher average temperatures confirmed to result into a somewhat lower mortality.

COVID-19 reported death rates for year 2020, virtually free from the effects of vaccination, are shown in Figure 2. Mean mortality in normal mean BMI countries was 4.8 (1755/369, Worldometer) and 13.7 (685/50, OWID) times smaller than that found in high mean BMI countries.

A view at the recognized major risk factor for death due to COVID-19, age, can be observed in Figure 3. Countries with longer life expectancies at birth were, as expected, hit the hardest. However, countries with a mean BMI <25 consistently showed a reduced mortality, which remained particularly low even among the few countries with the highest life expectancies.

The number of countries with a mean BMI <25 amounts to 27 (20.1% of the total of the 134 considered). The overall distribution of mortality in this group scales down to less than one fourth of that seen for BMI ≥25. The odds ratio between the odds of a lower mortality rate in presence of a BMI <25 and the odds of

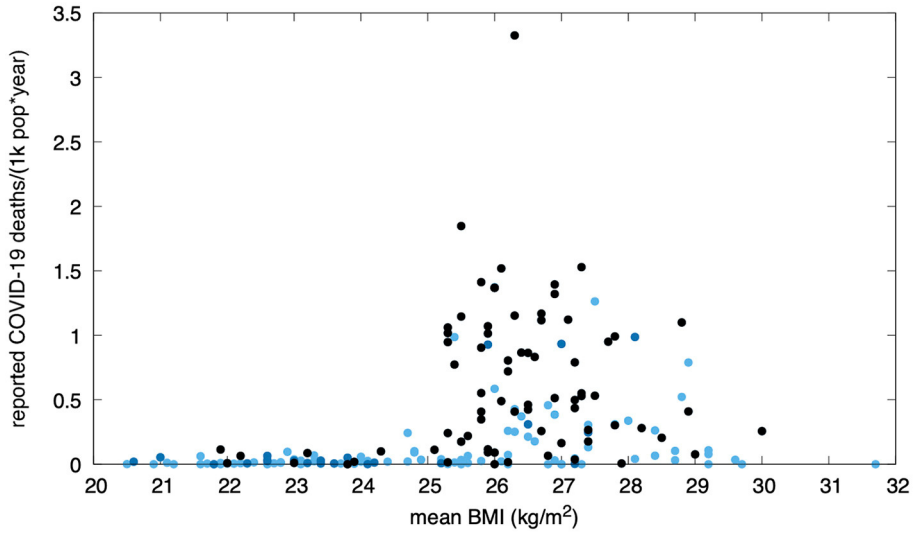

FIGURE 2
Pre-vaccination annual reported COVID-19 mortality in 183 countries (Our World in Data, year 2020) vs. mean BMI. Black dots correspond to countries who performed more than 50 tests per thousand people per year. Dark blue dots indicate countries who did not reach such a rate. Countries for which testing data was not available within this dataset are shown in light blue. The lower mortality rates among countries with a normal mean BMI can not be explained by undertesting alone (a few extensively-testing countries can be seen in this range).

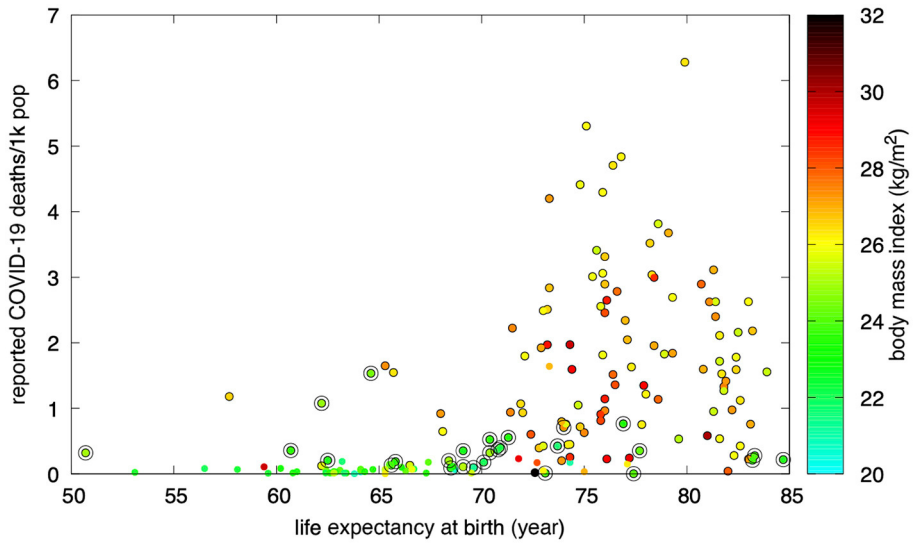

FIGURE 3
Countries with a normal mean BMI (circled) reported a lower COVID-19 mortality. Purportedly under-reporting countries are shown with edgeless dots.

a lower mortality rate in the absence of a BMI <25 is identically equal to 1.0 (24:24) when a reference value of 720 deaths per 1,000,000 population is taken. Defining "high reported mortality" as the mortality exceeding such a value, only 3 countries had high mortality in the presence of a BMI <25, against 83 countries with high mortality in the absence of a BMI <25. The resulting odds ratio was therefore 0.036 (3:83).

According to worldwide reported deaths due to COVID-19, high reported mortality in the presence of a mean BMI <25 was rare. Countries with a mean BMI <25 where the extent of testing was deemed representative showed less than one fourth the mortality observed in countries with a BMI ≥25.

Discussion

Two major limitations of this study are the uncertainties on undertesting and underreporting of deaths. Lower income countries typically tested less extensively and were less likely

to report deaths from COVID-19 with the needed accuracy. Actual mortality was higher than reported mortality in every part of the world, but the ratio between the two was correlated with income. Developing countries—the majority of which also have a lower mean BMI—had a much higher actual mortality compared to reported counts. This effect was, expectedly, less prominent in higher income countries. Although such a difference could have in principle represented a bias toward an overestimation of the effect of BMI on mortality, the absence of extensively-testing (high-income), normal-BMI countries reporting high mortalities remains.

Another limitation came from differential testing policy, which was not considered. However, although local and temporally delimited differences in country-specific decisions on testing symptomatic individuals only vs. everyone existed, governmental directions changed over time in most countries, making annual statistics less sensitive to this type of inequality.

As a simple anecdote, we would like to draw the reader's attention to the fact that a naked man walking in the rain in the winter is not unlikely to catch a cold. Although colds are often caused by a virus, we do not usually point the finger at the infective agent. We agree instead that the cause of his cold was his behavior, since he—aware of the risk—exposed himself to the elements. Indeed the use of face masks, social distancing and mass vaccinations, all offer protection against infection. However, limiting vulnerability to the new threats that day after day nature manages to assemble behind the scenes would not be inadvisable.

One potentially successful strategy, and a suggestion for an open medical ethics debate, would be that of entitling those who treat their body with more respect (such as normal-weight, non-smokers individuals) to small deductions on the contribution that in many countries patients owe for the cost of medical tests and treatments. An action of this kind has the potential to mitigate the obesity and smoking prevalence. It would simultaneously provide positive feedback to those who already look after their body, and would gradually awake the interest of those who struggle making an effort to change their attitude towards smoking and/or food. A rewarding scheme recognizing body respect would invert the current global trend of increasing obesity rates, simultaneously resulting in a significant reduction in public health expenditure.

Conclusion

A higher mortality from COVID-19—up to fourfold and over—was observed in many countries where the mean BMI exceeded the value of 25, while such increase was not seen in the few extensively-testing, normal-mean-BMI countries.

Caution should nevertheless be exercised in the interpretation of the reported correlations when attempting a causal analysis. Although both a coincidence and reverse causation can be excluded due to the correlation being statistically significant and temporally unidirectional, mean values do not contain information about distribution. A country with a bimodal distribution of BMI (for example were many people are underweight and obese) could have a similar mean BMI of a country were most people have a normal BMI. Finally, a high BMI does not represent neither a necessary nor a sufficient condition for death due to COVID-19. Common causation can not be ruled out.

As with any morphometric index, BMI comes with issues. International variations are well known, such as a reduced upper normal value for Asians (38). A more promising index seems to be the dimensionless waist-to-height ratio, in short WHtR, for which at the time of this writing a global report is still missing.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RG wrote the manuscript, retrieved the data, and prepared the figures. NMP revised the manuscript, analyzed data comparability criteria, encouraged discussions, and elaborated on correlates. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1106313/full#supplementary-material

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Potential biocide roles of violacein

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Violacein is a pigment produced by Gram-negative bacteria, which has shown several beneficial biological activities. The most relevant activities of violacein include the interference in the physiological activities of biological membranes, inhibition of cell proliferation, antioxidant, and anti-inflammatory activities. Moreover, the antiviral activities of violacein against some enveloped and non-enveloped viruses have also been reported. Violacein showed a wide spectrum of protease inhibition, both experimentally and *in silico*. Other *in silico* studies have suggested that violacein binds to the SARS-CoV-2 spike. Empirical physicochemical studies indicate that violacein (or, occasionally, its derivatives) may be administered orally to treat different disorders. In addition, different alternatives to product violacein, and molecular devices for delivery of this pigment are reviewed.

KEYWORDS

violacein, COVID-19, membrane interaction, antioxidant activity, anti-inflammatory activity, protease inhibition, violacein antiviral activities

1 Introduction

In the last five decades, viral infections have negatively affected the world population, mainly because of accelerated globalization and rapid urbanization, which are associated with strong changes in social habits. Many viral infections have spread worldwide through different vectors, such as dengue (DENV), Ebola, Hepatitis B virus, herpes virus, HIV/AIDS, Middle East respiratory syndrome, rotaviruses, and Zika. Recently, the global population has been in peril with the SARS-CoV-2 pandemic. The world death toll of SARS-CoV-2 overpassed 6.8 million people worldwide, and long-term deleterious consequences have been described in some of the 755 billion infected people based on the World Health Organization (WHO) website (WHO, 2023). Different therapeutic strategies have been developed worldwide for the treatment and prevention of SARS-CoV-2 infection. Many old and traditional antiviral drugs were on the battlefront trying to stop the viral pandemic but with poor or disappointing results (Das et al., 2020). The processes to develop SARS-CoV-2 vaccines, get the corresponding approval, and deliver them worldwide took approximately six to 12 months. Although large-scale worldwide vaccination has substantially reduced the death toll, new viral variants still challenge the effectiveness of marketed vaccines making their production more complex. Effective drug treatments are, however, still needed to complement vaccine development, especially for unvaccinated

people or groups that may not appropriately respond to vaccination (such as immunocompromised patients), and to cover viral mutations not covered by available vaccines (Mei and Tan, 2021).

Nature provides an enormous number of secondary metabolites with a wide variety of molecular scaffolds produced from different biological kingdoms. Among secondary metabolites, pigments have been used in many applications since the beginning of human society and are presently employed in many industrial areas, such as cosmetics, textiles, foods, and healthcare (Kulandaisamy et al., 2020). Microorganisms are one of the relevant pigment producers because they can be cultured under controlled conditions, have simple nutritional requirements, fast and reproducible growth, are easy to scale up, and provide well-established purification methods that are available in the market; they are also environmentally friendly (Mumtaz et al., 2019). *In silico* screening is among the modern approaches to find novel drug candidates; in particular, structure-based virtual screening departs from experimental or *in silico* predicted protein structures to select from large public chemical databases or relatively small in-house libraries, those compounds that are more likely to bind to a known or putative binding site (Rahman et al., 2022). Virtual screening has been extensively applied to find potential therapeutics against COVID-19. For example, using this approach 26 synthetic derivatives of coumarins and quinolines were analyzed by molecular docking and molecular dynamics; among them, six compounds were predicted to possess high binding capacity against SARS CoV-2 main protease (M^{pro}) (Yañez et al., 2021).

Several microbial secondary metabolites are currently marketed for therapeutic applications that started at the beginning of the last century with penicillin, diverse anthracyclines, mitomycins, etc., and have recently been reviewed (Abdelghani et al., 2021). Since the sanitary SARS-CoV-2 emergency, antiviral applications of many molecules and pigments, and their biological mechanisms have recently been revisited (Azman et al., 2018; Ma et al., 2020; Selim et al., 2021).

Violacein has recently attracted the attention of researchers owing to its wide variety of biological activities. During the last two decades, several reports have described numerous biological activities of this pigment, including immunomodulatory, antimicrobial, antiparasitic, antifungal, anticancer, and antiviral activities (Duran et al., 2021a; Duran et al., 2021b; Duran et al., 2022). The relevance of the antioxidant properties of violacein must be analyzed in the context of COVID-19 pathology, as acute infections of SARS-CoV-2 can produce cell death and long-term neurological pathologies. In general, most viral infections are related to the reduction of antioxidant physiological pathways mainly by the inhibition of Nuclear Factor Erythroid 2 (NRF2), a transcription factor (*i.e.*, leucine zipper), which triggers antioxidant proteins and hampers the NLRP3 inflammasome, which mediates the release of many cytokines (Zhu et al., 2021). Additionally, SARS-CoV-2 induces activation of Nuclear Factor kappa B (NF-κB), promoting inflammation and oxidative stress. The direct consequence of SARS-CoV-2 is the development of elevated levels of inflammation and generalized oxidative processes. These processes are developed by the activation of pro-inflammatory cytokines (*i.e.*, Tumor Necrosis Factor-alpha o TNF-α, IL-1β, and IL-6) produced by macrophages and monocytes, high recruitment of immune and endothelial cells, and platelets (Tay et al., 2020). The NF-κB activation induces high activities of cyclooxygenase 2 (COX2) and NOX2 (NADPH oxidase) responsible for ROS production and mitochondrial oxidative stress. Some of the postulated molecular mechanisms of SARS-CoV-2 infection have recently been reviewed (Chernyak et al., 2020). Besides, COVID-19 is not only considered a respiratory viral disease, but is also associated with endotheliopathy, triggering many molecular markers such as angiopoietin 2 plasminogen activator inhibitor 1 (PAI-1) and Willebrand factor (vWF), among others, and compared to healthy individuals. These factors can be associated not only with fatigue and circulatory diseases (*i.e.*, immune thrombosis and myocardial infarction) but also with neuropsychiatric pathologies (*i.e.*, cognitive disorders and stroke) in acute COVID-19 infections and long-term COVID (Laforge et al., 2020; Fodor et al., 2021; Li et al., 2021). A recent study conducted in Nigeria showed that the levels of glutathione; vitamins A, C, and E; enzymes with antioxidant activities such as superoxide dismutase, catalase, and glutathione peroxidase; and the concentrations of Cu, Mg, Se, and Zn are lower in patients with COVID-19 than in healthy people (Muhammad et al., 2021). Intravenous administration of vitamin C, a well-known antioxidant compound, at high concentrations ameliorates inflammation and oxidative stress in patients with severe COVID-19 (Vollbracht and Kraft, 2022). COVID-19 pathology mediated by inflammation associated with the oxidative stress cascade unquestionably contributes to disease severity. Several trials of potential therapeutic antioxidant molecules to treat SARS-Co-2 infection are in progress in the US (https://clinicaltrials.gov/).

It is of particular interest to analyze the antiviral activities of violacein against viruses since the emergence of SARS-CoV-19 is correlated with the scarce activity of many therapeutic molecules.

The present work endeavors to describe the potential mechanisms and properties of violacein as a potential antiviral agent against SARS-CoV-2. Analyses of the effects of violacein on cellular membranes, and antioxidant, anti-inflammatory, and antiviral activities are overviewed (Figure 1). Studies on protease inhibition and *in silico* studies have also been summarized. Finally, alternatives for the production and delivery of violacein are discussed.

2 Violacein properties and biological activities

2.1 Violacein general properties

Violacein is a low molecular weight (MW = 433.41 g/mol) bacterial purple pigment produced mainly by Gram (−) bacteria and has been recently reported (Duran et al., 2021a). *Chromobacterium* spp. and *Janthinobacterium* spp. are the most common violacein producers. (Duran et al., 2021b).

The IUPAC formula for violacein is 3-[2-hydroxy-5-(5-hydroxy-1H-indol-3-yl)-1H-pyrrol-3-yl]indol-2-one ($C_{20}H_{13}N_3O_3$). From a structural point of view, violacein is a bisindole molecule composed of three heterocycles and a 2-pyrrolidone molecule that links two indoles, an oxindole, and 5-hydroxyindole (Figure 2).

Violacein is a very hydrophobic molecule characterized by a P of 2.7 (PubChem) and Log Po/w = 3.34 (Choi et al., 2020).

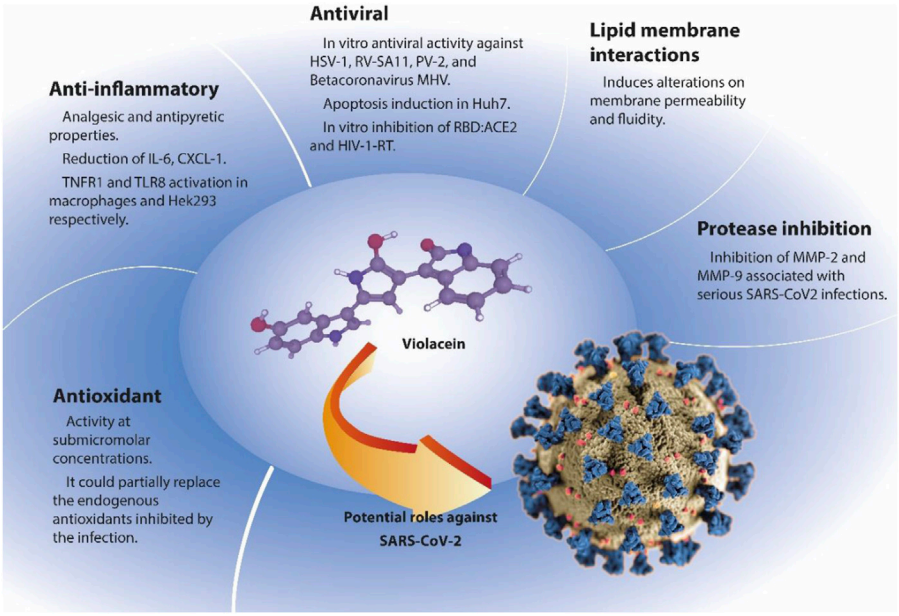

FIGURE 1
Potential antiviral violacein activities. Example: SARS-Cov-2. Abbreviations: IL-6 (Interleukin 6), CXCL-1 (chemokine ligand 1), TNFR1 (Tumor necrosis factor receptor 1), TLR8 (Toll-like receptor 8), HSV-1 (Herpes simplex virus 1), RV-SA11 (Simian rotavirus SA11), PV-2 (Poliovirus type 2), MHV (Murine hepatitis virus), Huh7 (human hepatocellular carcinoma cell line, which contains complete genome replic*on of Hepatitis C virus*), RBD:ACE2 (*receptor-binding domain to angiotensin converting enzyme 2 [ACE2]*), HIV-1-RT (human immunodeficiency virus *-1 reverse transcriptase*), MMP-2 and MMP-9 (*matrix metalloprotease 2 and 9 respectively*). Coronavirus COVID-19 structure illustration was created at the Centers for Disease Control and Prevention (CDC). Modified from the CDC, Alissa Eckert, MS; Dan Higgins, MAM (Public Domain).

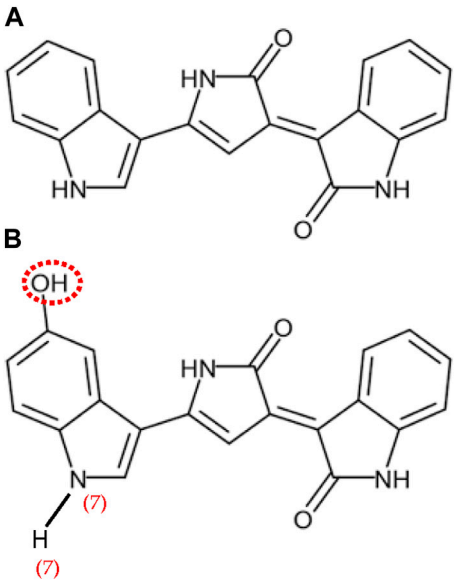

FIGURE 2
Chemical Structure of deoxyviolacein **(A)** and **(B)** violacein, adapted with permission from Nelson Durán et al., licensed under CC BY-SA 4.0.

Violacein is insoluble in aqueous media but is partially soluble in organic solvents such as acetone and dioxane, less soluble in ethanol and propanol, and soluble in ethyl acetate, DMSO, and methanol.

The UV-Visible spectra of violacein showed two maximum peaks at λmax = 577–585 nm depending on the organic solvent, and a UV peak at λ_{max} = 260.3 nm in methanol (Abboud and Arment, 2013).

Violacein biosynthesis involves four genes arranged in a gene cluster vioABCD of 8 kb. The synthesis of violacein involves the condensation of two L-tryptophan molecules at the oxindole position, followed by intramolecular reorganization at the 5-hydroxy-indole ring (1→2 shift) (Duran et al., 2021a).

The physicochemical properties of violacein were analyzed in relation to some typical empirical rules, namely: Lipinski and Veber (which predict whether a drug like compound is likely to be orally bioavailable via passive absorption) (Lipinski et al., 2001; Veber et al., 2002) and Pfizer 3/75 rule (which suggests that a drug candidates in the chemical space of low calculated log P and high topological surface area are not likely to cause significant toxicological effects at total plasma concentrations below 10 μM) (Hughes et al., 2008). Violacein fulfills all of them, thus being a good candidate for oral administration with likely low toxicity (Supplementary Table S1).

2.2 Violacein antioxidant activity

The antioxidant activity of violacein was reported for the first time in 1998 and confirmed by several extensive studies by Duran's research group during the 2000s and recently reviewed (Duran et al., 2021a; Durban et al., 2021b). The presence of several conjugated

double bonds and two peaks in the UV-visible spectra suggests a protective effect against visible and UV radiation. The experimental antioxidant activity of violacein has been confirmed by theoretical studies based on an electron density model and spectroscopic analysis (Cao et al., 2007; Jehlička et al., 2015). The estimated ionization potential (IP) of violacein is 146.88 kcal/mol which is about 5% lower than a well-known antioxidant *a*-tocopherol (IP = 154.90 kcal/mol). The antioxidant activity of violacein has been attributed to the N7-H7 band of 5-hydroxyindole (Cao et al., 2007).

In 2006, violacein antioxidant activity against lipid peroxidation was experimentally evaluated in three models of lipid membranes, including egg and soybean phosphatidylcholine liposomes, and also in rat liver microsomes. Protection of lipid membranes by violacein, either in solution or reconstituted within the liposomes, was evaluated against nitric oxide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and ascorbyl radicals in the presence or absence of the biodye showing IC_{50} values of 21, 30, and 125 μM, respectively (Konzen et al., 2006). It was also observed that reconstitution of violacein into the liposomes enhanced its antioxidant activities. Liposomes in the absence of violacein were used as control. A more recent *in vitro* study on violacein antioxidant properties evaluated violacein partially purified from the isolate *Chromobacterium vaccinii* CV5 against common radicals. The IC50 of violacein against DPPH, superoxide (produced from phenazine methosulphate), nitric oxide (produced from sodium nitroprusside), hydrogen peroxide, and hydroxyl (produced from ferrous ammonium sulfate) were 0.87 μM, 0.91 μM, 1.19 μM, 0.86 μM, and 0.85 μM, respectively (Vishnu and Palaniswamy, 2018). The lower *in vitro* IC_{50} values reported in this study could be attributed to the partial purification of violacein. In any case, all these results suggested a protective effect mediated by violacein at micromolar or sub-micromolar concentrations against peroxidation produced by radical species.

In another study, the antioxidant activity of violacein produced by *C. violaceum* wild-type and mutants less- and non-biodye producers (*i.e.,* CV9, CV13, and CV14, respectively) was analyzed (Abboud and Arment, 2013). All *C. violaceum* strains were UV-irradiated at λ = 253.7 nm with 6,000 μW s^{-1} cm^{-2}. Non-violacein-pigmented mutants did not grow, whereas the viability of the wild-type strain and violacein hyper-producer mutants was reduced by UV irradiation for 48 h. These results suggest the potential protective effects of Violacein against DNA UV-induced damage. Additionally, catalase activity in non-violacein-producing strains was enhanced compared with the enzymatic activity of violacein hyper-producer *C. violaceum* strains. This experiment strongly suggested an active scavenging role of violacein against reactive oxidative species (ROS). Violacein has been added to sunscreens to increase its protective effect against potential UV damage (Suryawanshi et al., 2015).

2.3 Violacein anti-inflammatory activity

The analgesic, antipyretic, and immunomodulatory effects of violacein produced by the newly isolated *C. violaceum* ESBV4400 were evaluated in Wistar albino rats and mice (Antonisamy and Ignacimuthu, 2010). The effects of violacein against non-inflammatory and inflammatory pain, anaphylactic reactions and fever were assayed by injecting with ovalbumin, acetic acid, formalin, sheep red cells, and *Saccharomyces cerevisiae* yeast to create traumatic trials. The harmful effects in the treated animals were countered by using indomethacin, dexamethasone, disodium cromoglycate, naloxone, and morphine as control drugs. In the delayed hypersensitivity and provoked paw anaphylaxis tests employing red blood sheep cells and ovalbumin. Similar results were observed with 10 mg kg^{-1} dexamethasone paired with 5 mg kg^{-1} disodium cromoglycate and 40 mg kg^{-1} violacein, with less than 2% difference. In the case of severe pain induced by acetic acid, the results showed a 93.9% and 78% reduction in writhing using 5 mg kg^{-1} and 10 mg kg^{-1} morphine and indomethacin. Similar response was elicited by 40 mg kg^{-1} violacein. Comparable results were obtained using the formalin assay. Hyperthermia induced by the yeast *S. cerevisiae* injection in rodents showed normalization of temperature with 150 mg kg^{-1} paracetamol after 60 min, and a similar response was observed with 20 mg kg^{-1}, and 40 mg kg^{-1} violacein after 120 min and 60 min, respectively. The authors concluded that violacein exerts an immunosuppressive effect on inflammatory physiological responses through T Cells, antiallergic activity to the anaphylactic Ig E-mediated response, and antipyretic response by inhibiting prostaglandin synthesis.

In another report, the effect of violacein on acute and chronic inflammation in mice (C57BL/6) produced by intraperitoneal injection of 1.0 μg LPS from *Escherichia coli* 0111:B4 was analyzed (Verinaud et al., 2015). Dendritic cells and their CD80 and CD86 markers treated with LPS and violacein did not show variations compared to control cells. Comparable results were obtained for B- and T-cells, which did not show signs of toxicity. In addition, mice treated with LPS-violacein exhibited a significant decrease in neutrophil infiltration in the peritoneal cavity compared to control mice. In addition, cytokines IL-6 and CXCL-1 were reduced, whereas IL-10 was increased in the serum. An Experimental Autoimmune Encephalomyelitis mouse model was developed by induction with myelin oligodendrocyte glycoprotein (MOG35–55). The authors reported an increase in scurfin protein levels (FoxP3, considered a primary regulator of T regulatory cells (Tregs) in mice treated with violacein.

A cellular study using immune lineages of human peripheral blood mononuclear cells (PBMCs) and two murine macrophage cells (ANA-1 and Raw 264.7, established by retroviral infections) treated with violacein was carried out (Venegas et al., 2019). Macrophages exposed to violacein displayed elevated levels of pro-inflammatory TNFα concomitant with TNF receptor 1 (TNFR1) activation, which induces cellular apoptosis. In addition, the authors suggested that Toll-like receptors (TLRs) could be activated by violacein in murine cells, which are strongly associated with PAMPs (*i.e.*, pathogen-associated molecular patterns). Particularly, human TLR8 was activated by violacein in the transfected HEK-293 cell line, and an *in silico* docking proposed a binding mode of violacein to the receptor similar to the antiviral drug imidazoquinolinone (Venegas et al., 2019). Members of the imidazoquinolines and imidazoles, which resemble the heterocycle structure of violacein, are known to activate TLR7/8, triggering cytokine production by the immune system associated with the clearance of virally infected cells and treatment of skin cancer. The activation of TLR7/8 by

imidazoquinolines and imidazoles depends on the electronic configuration of the heterocycles (Kaushik et al., 2021).

Briefly, the major anti-inflammatory effects of violacein were associated to the reduction of pro-inflammatory cytokines such as TNF-α, TGF-ß, IL-1β, and IL-6, and chemokines CXCL1 and CXCL12 involved in the cell recruitment but also increase the expression of IL-4 and IL-10 (Antonisamy and Ignacimuthu, 2010; Platt et al., 2014; Verinaud et al., 2015).

2.4 Violacein antiviral activities

In a pioneering study, May et al. (1989), May et al. (1991) reported the *in vitro* antiviral activity of violacein/deoxyviolacein (90/10) in HeLa cells infected with poliovirus and herpes simplex virus (HSV-1). The IC_{50} value of violacein against HSV-1 infected cells was estimated to be 0.577 μM. A decade later, another study challenged the *in vitro* antiviral activity of violacein against HSV-1, Simian rotavirus SA11 (RV-SA11), and Poliovirus type 2 (PV-2) strains with pigment concentrations approximately 1/3–1/2 below the cytotoxicity levels on the tested cell lines (*i.e.*, HEp-2, FRhK-4, MA104, or Vero cell cultures). The antiviral activity of 1.25 μM violacein against the tested virus by the MTT assay ranged from 24.3% to 8.5%, depending on the virus and strain (Andrighetti-Fröhner et al., 2003).

The differences in violacein effectiveness against the tested viruses entail analyzing the main characteristics of the viral surface to underpin the potential antiviral mechanism of the pigment. Rotaviruses are non-enveloped viruses that contain a glycoprotein, VP7, attached to the cell surface. Glycoprotein VP7 is composed of 326 amino acids, with two hydrophobic domains in the amino-terminal group (Poruchynsky et al., 1985). In addition, polioviruses are non-enveloped viruses with three main proteins, VP1, VP2, and VP3, on the capsid surface, that are folded in a hydrophobic ß-barrel (He et al., 2000). On the other hand, members of the herpes virus family are enveloped, having a lipid membrane structure obtained from the host covering the virion. Similarly, viral structures can be observed in coronaviruses and human immunodeficiency virus (HIV). Despite the different surface characteristics of the three tested viruses, all shared hydrophobic moieties on their surfaces that could interact with water-insoluble molecules, such as violacein. Since the interaction of violacein with molecules with hydrophobic motifs (*i.e.*, non-ionic surfactants, cyclodextrins, aromatic ionic liquids, lipid carriers) were previously reported (de Azevedo et al., 2000; Rivero Berti et al., 2019; Rivero Berti et al., 2020; Rivero Berti et al., 2022). It is expected that hydrophobic interactions appear to be the unspecific major mechanism of interaction between the virus and violacein.

Murine hepatitis virus (MHV), like SARS-CoV-2, belongs to the genus Betacoronavirus. Both are enveloped, with glycoproteins on the surface and a genome composed of positive-sense single-stranded RNA within a nucleocapsid. The inhibition of MHV-3 in L929 (mouse fibroblasts, ATCC CCL-1) infected cells by 20 μM violacein was 42% after incubation at 37°C for 1 h (Gonçalves et al., 2023).

In a recent physicochemical study, the *in vitro* inhibitory effects of purified violacein and deoxyviolacein on CoV-2 spike RBD: ACE2 and HIV-1 Reverse transcriptase proteins were analyzed (Supplementary Table S2). These results indicated that deoxyviolacein had a low inhibitory effect on both proteins at millimolar concentrations. Meanwhile, 1 mM violacein inhibited 94.3% of HIV-1 Reverse transcriptase, and CoV-2 spike RBD: ACE2 was inhibited by 53% at 2 mM violacein (Dogancı et al., 2022). The concentration of the pigment needed to inhibit the activity of these proteins is approximately one thousand times higher than the high levels required to induce cytotoxicity in most of the reported cellular cell lines (de Sousa Leal et al., 2015; Duran et al., 2021a).

The low antiviral activity of violacein against HSV-1, Simian rotavirus 219 SA11, and Poliovirus type 2 reported previously (Andrighetti-Fröhner et al., 2003) could be attributed to its extremely poor solubility of the biodye under physiological environmental conditions, which may be a major obstacle to replicate the conditions of *in vitro* assays (*e.g.*, the concentration of the dye) in cell assays or *in vivo*. The insolubility of violacein in aqueous media can be attributed to the lack of polar groups and the presence of aromatic motifs. The interaction of indole aromatic residues of different molecules that can pile on each other, which is attributed to π-π stacking, reduces violacein interactions with other molecules, including water. Consequently, molecular aggregates of violacein display reduced biological activities, similar to some low-solubility marketed drugs containing aromatic rings (Islan et al., 2012). It would be of most interest to study whether it is possible to generate violacein derivatives that retain or even potentiate the molecular interactions responsible for its antiviral effects (increasing potency) and, simultaneously, include structural modifications that diminish their π-π stacking potential, reducing their tendency to aggregate. It is important to bear in mind, however, that π-π stacking can be exploited in the design of last-generation drug delivery systems, including high purified carbon- or graphene-based nanomaterials, nanocomposites, targeted delivery systems, and prodrug delivery systems such as self-assembled polyprodrug amphiphiles (Zhuang et al., 2019). The key question appears to be: Can violacein molecular structure be modified in a way that the derivatives retain the ability to interact with viral constituents (which, as explained later, also requires π-π stacking) and load the dye into state-of-the-art drug delivery systems, and at the same time display enhanced aqueous solubility? Are these mutually exclusive properties? In any case, fine-tuning would likely be required at the molecular optimization step.

Besides that, the antiviral mechanisms of violacein were not reported yet, based on the biophysical properties of the biodye it could be speculate that the hydrophobic interaction between the virus moieties and violacein will be the major driven force for virus inactivation.

2.5 Violacein and membrane interactions

Enveloped viruses are causative agents of diverse infections. Enveloped viruses have a lipid membrane covering the viral structure (*i.e.*, the capsid) that originates from the infected host. Among the advantages of encapsulated viruses, the fusion of the viral membrane with the cell membrane is considered to be one of the most effective strategies for infecting cells. In particular, many coronaviruses have a bilayer of lipids and proteins, forming an

envelope around their capsids. This envelope is derived from host membranes during virion formation but also plays a crucial role during infection when virus particles adhere to the host cell membrane (Nardacci et al., 2021). Therefore, the role of lipids in SARS-CoV-2 infection and pathogenesis cannot be underestimated. In a previous study, the existence of pockets of high hydrophobicity in the SARS-CoV-2 protein S (spike), which firmly bind linoleic acid, was described. This binding seemingly locks the S protein in a state that decreases its association with the ACE2 receptor (Toelzer et al., 2020). These types of hydrophobic pockets have previously been used to develop drugs to treat rhinoviruses (Casanova et al., 2018).

Additionally, lipid membranes play a key role in the replication of all RNA (+) viruses, including coronaviruses. These viruses manipulate the host membranes to form viral replication organelles. In addition to sequestering the cellular machinery necessary for viral multiplication, these organelles may also play a role in evading the immune response. It is known that coronaviruses alter the cellular metabolism of lipids, favoring the synthesis of sterols and fatty acids that are propitious to them in an analogous way to that in which tumor cells reprogram lipid metabolism to ensure their survival (Borella et al., 2022; Rudiansyah et al., 2022). Furthermore, coronaviruses interfere with exosome formation, autophagy, and lipid rafts (Casari et al., 2021). This reorganization of lipid metabolism and membrane mechanisms ensures or promotes viral multiplication and is, therefore, a potential target for new drugs or therapeutic strategies.

Therefore, studies of violacein-related lipids and membrane systems are of interest. While violacein does not have a typical amphiphilic head-tail structure, it is hydrophobic and has some polar groups; therefore, direct interaction with cell or viral membranes is highly probable (de Souza et al., 2017). Cauz *et al.* (2019) described some interactions between violacein and membranes, initially, to explain its antimicrobial activity. However, these findings may be useful in explaining its antiviral activity. *In vitro* experiments have shown that the addition of violacein to large unilamellar vesicles (LUVs) disrupts these vesicles and produces leaks, thereby affecting their permeability. Additionally, because of the low concentration of violacein required to produce these effects, they hypothesized that violacein does not act as a tensioactive, like many antimicrobials, but rather produces defects and discontinuities in phospholipid organization that alter membrane permeability (Cauz et al., 2019). This partially contrasts with previous results; de Souza *et al.* (2017) explored violacein interactions with phospholipid thin films to model eukaryotic plasma membranes and found that violacein effectively interferes with the correct ordering of phospholipids, affecting their compressibility and viscoelasticity, and therefore fluidity, but does not produce changes in permeability in the tested models (de Souza et al., 2017). In a recent study, the effect of violacein on synthetic monolayer lipid membranes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-ethylphosphocholine chloride salt (DSEPC), and 1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DPPG) was studied using the Langmuir-Blodgett technique. The isotherms of violacein in the cationic and zwitterionic lipid monolayers showed a decrease in lipid molecular area, suggesting compaction. However, compaction was not observed in negatively charged lipid monolayers. Further analyses with X-rays indicate that violacein decreases the lipid tilt angle, which consequently induces the thickening of lipid monolayers (Gupta et al., 2021; Gupta and Ghosh, 2023). These results indicate the interaction of the aromatic electrons of violacein with the positive residues of lipid-like π-σ non-specific molecular interactions.

A recent study showed that the release of violacein from the cells in one of the main bacterial pigment producers, *Chromobacterium violaceum* (ATCC 12472), occurred through approximately 100 nm extracellular membrane vesicles. The study revealed that 79.5% of the violacein encapsulated in the membrane vesicles remained in solution, and the membrane vesicles provided an increase of violacein estimated in 1740 folds (Choi et al., 2020). The strategy of violacein release in *C. violaceum* possesses two relevant characteristics: first, the pigment will not be attached to the cell surface because the bacteria grow in aqueous media and violacein is a very hydrophobic molecule; and second, the released membrane vesicles can be attached to the hydrophobic surfaces of several molecules and structures, such as the VP7 glycoprotein of rotaviruses and the *ß*-barrel structure of VP1-VP3 of poliovirus. The interaction of viral glycoproteins present on the virus surface handles virus-cell interactions, leading to causative infections (Cosset and Lavillette, 2011). Consequently, membrane vesicles containing violacein can easily merge with other membranous lipid structures of enveloped viruses (HSV-1, SARS-CoV-2, HIV, etc.).

The main cellular target of violacein are biological membranes and molecules because of violacein hydrophobicity (*i.e.*, low solubility in watery environments). Additionally, the recent finding of violacein which is released from the producer cells in the form of membranous structures will favor the interaction within mammalian cell membranes and also facilitates the entrance of violacein to the cytoplasm.

2.6 Violacein inhibition activity on proteases

The traditional mechanism of SARS-CoV-2 infection involves the binding of the spike (*i.e.*, S glycoprotein) to ACE2 (*i.e.*, angiotensin-converting enzyme type 2) to produce a membrane fusion by a serine protease (*i.e.*, TMPRSS-2) or by the cysteine protease pathways (*i.e.*, cathepsins). In recent studies, the spike in SARS-CoV-2 variants was found with a wider spectrum of action by activating the transmembrane serine protease 13 (*i.e.*, TMPRSS-13) and the matrix metalloproteases 2 and 9 (*i.e.*, MMP-2 and MMP-9). High MMP levels were found in patients with serious SARS-CoV-2 infections. Supporting the role of proteases in viral infection, protease inhibitors can reduce the infection of the SARS-CoV-2α in the human fibrosarcoma HT1080 cell line (Benlarbi et al., 2022).

In previous work, the inhibition of matrix metalloproteases (MMPs) by violacein was reported (Platt et al., 2014). The authors reported that violacein inhibited MMP-2 in the MCF-7 cell line, and the metalloprotease played a relevant role in the secretion of CXCL12, an inflammatory cytokine. Additionally, violacein inhibited MMP-9, which downregulates the *a*-chemokine receptor (CXCR4) specific for stromal-derived factor-1, a chemotactic molecule for the recruiting lymphocytes (Platt et al., 2014).

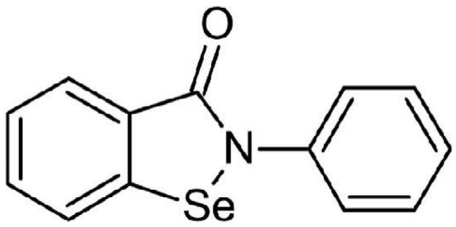

FIGURE 3
Ebselen molecular structure, reprinted from Benjah-bmm 27, Public Domain, via Wikimedia Commons

Recently it was suggested that violacein presumably acted as a protease inhibitor against the ACE2 receptor, and as an immunomodulator against COVID-19 (Durán et al., 2022). M^{pro}, also called $3CL^{pro}$, and also the papain-like protease (PL^{pro}) is responsible for viral polyprotein disruption, a process important for the virus to survive and replicate. M^{pro} is a decisive coronavirus enzyme that plays essential roles in modulating two key processes: viral transcription and replication, which makes it an attractive pharmacological target against SARS-CoV-2.

Then, one strategy could be protease inhibition as a target to M^{pro}, once it was shown that inhibition of many proteases was discovered by researching the cytotoxic actions of violacein. Decease caused in CD34+/c-Kit+/P-glycoprotein+/MRP1+ TF1 leukemia progenitor cells was moderated by calpain (calcium-dependent protease-cysteine protease) suppression and by decease-associated protein kinase 1 (DAPK1). Comparative analysis showed that violacein also induced several protein kinases activities, such as protein kinase A (PKA), pyruvate dehydrogenase kinase (PDK), and protein kinase B (AKT), that were monitored by structural modifications induced by endoplasmic reticulum stress and Golgi apparatus collapse, as led to cell decease (Queiroz et al., 2012). It is attainable concluding some chemical structural comparison between violacein activity and that of protease suppressors such as Ebselen (Figure 3).

Following the suppression data, the HIV-1 RT suppression rate of violacein (1 mM) from the *Janthinobacterium* sp. GK strain was higher than 90%, and the SARS-CoV-2 spike RBD: ACE2 suppression rate of violacein (2 mM) was higher than 50%. *In silico* studies were performed to explore the potential interactions among deoxyviolacein and violacein and three reference compounds with the target proteins: ACE2, SARS-CoV-2 spike RBD (*i.e.*, receptor binding domain), and HIV-1 RT (*i.e.*, reverse transcriptase). Violacein seems to bind strongly to the three receptors as showed by their docking binding energies: −9.94 kcal/mol for ACE2, −9.32 kcal/mol for HIV-1 RT, and −9.32 kcal/mol for SARS-CoV-2 spike RBD. Comparable results were obtained for deoxyviolacein: −10.38 kcal/mol for ACE2, -9.50 kcal/mol for HIV-1 RT, and −8.06 kcal/mol for SARS-CoV-2 spike RBD. Following these outcomes, deoxyviolacein and violacein seem to bind to all the receptors with high efficiency. HIV-1-RT and SARS-CoV-2 spike protein suppression searches with deoxyviolacein and violacein were reported for the first time in the literature (Doganci et al., 2022).

The inhibition of diverse proteases involved in the viral infection process by violacein observed experimentally and by *in silico* molecular docking is a relevant advantage from therapeutic point of view because it is additional feature to the many biological activities of violacein which will reduce the chance of drug multi-resistance displayed by many molecules available in the market.

2.7 Violacein cytotoxicity

In vitro cell toxicity of violacein was evaluated in four mammalian cell lines (*i.e.*, Vero, MA104, FRhK-4, and HEp-2) using cell morphology by light microscopy, cell viability by Trypan blue and by the MTT assays (Andrighetti-Fröhner et al., 2003). The violacein cytotoxic concentration 50% was in the range of 2.07 a 3.55 μM, which depends on the used technique and the cell line. While the concentrations of violacein showed the best antiviral activity at 1.25 μM against HSV-1 (*i.e.*, strains KOS, VR-733, RV-SA11) with inhibition percentages in the range of 17.75%–24.27% and compared with acyclovir with inhibition percentages close to 100% Besides, the antiviral violacein concentration assayed was 35.5 to 8.9 times lower compared to the acyclovir (Andrighetti-Fröhner et al., 2003). In general, violacein cytotoxicity in non-tumoral cell lines is in the range of 5 μM–10 μM, meanwhile tumoral cell lines are more sensitive to the biodye with a cytotoxic range of 0.71 μM to about 5 μM (de Sousa Leal et al., 2015; Rivero Berti et al., 2020). Several mechanisms of violacein cytotoxicity including disturbance of mammalian cell membrane and polarization of mitochondrial membrane, disruption of actin function, p44/42 mitogen activated protein kinase (MAPK), apoptosis mediated by caspase 3, etc. were reviewed recently (Duran et al., 2016; Durban et al., 2021a). Besides, it is currently accepted that violacein displays low toxicity *in-vitro* against non-tumoral cell lines.

2.8 Violacein *in silico* binding studies

In an attempt to further study the potential binding site of violacein within the SARS-CoV-2 Spike protein, five potential binding sites reported in the literature were explored through molecular docking using AutoDock4 (Morris et al., 2009). The binding sites analyzed in this study are shown in Figure 4. Two sites are located within the S2 subunit: site 1 refers to the arbidol site previously published (Shuster et al., 2021) and site 2 corresponds to the fusion peptide binding site described in a recent report (Hu et al., 2021). The other sites are located within the S1 subunit: site 3 is a potential allosteric site reported by Wang *et al.* (2022), site 4 corresponds to the experimentally determined biliverdin site in the N-terminal domain (NTD) (Rosa et al., 2021), and site 5 addresses two possible reported sites in the RBD subunit, which are the Spike: ACE2 protein-protein interaction (PPI) interface (site 5α) (Bojadzic et al., 2021) and the site reported by Dogancı et al. (2022) for violacein from the blind docking study on the isolated RBD subunit (site 5β). In addition, the entire RBD subunit was considered for the docking calculations to explore other possible binding sites within such subunit (site 5γ). The biliverdin site has not been considered an inhibitor site, because biliverdin binding helps Spike evade antibody immunity (Rosa et al., 2021). Nevertheless, it was decided to include it in the present study

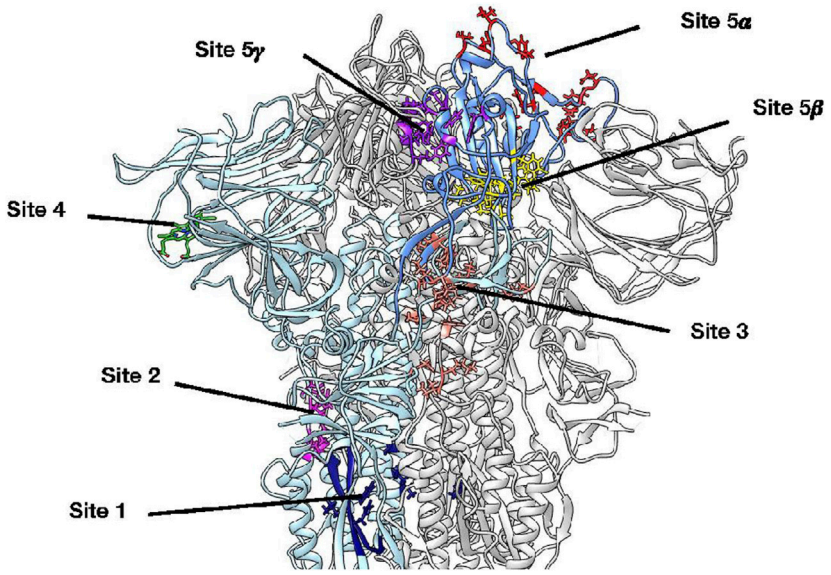

FIGURE 4
3D structure of the SARS-CoV-2 Spike protein, where all potential violacein binding sites studied by molecular docking are highlighted on protomer A. Protomer A is colored light blue while the corresponding RBD subunit is cornflower blue. Site 1 (navy blue) refers to the arbidol site located within the S2 subunit, site 2 (magenta) corresponds to a fusion peptide site, site 3 (salmon) is a potential allosteric site within the S1 subunit, site 4 (lime green) corresponds to the biliverdin site in the NTD, site 5α (red) refers to the Spike: ACE2 PPI interface, site 5β (yellow) corresponds to the site reported by Dogancı et al. (2022) for violacein from a blind docking study on the RBD subunit, and site 5γ (purple) emerged by considering the entire RBD subunit for docking calculations; to the best of our knowledge, this is the first report on this putative binding site.

because it is an experimentally validated binding site for small molecules.

The docking results obtained are shown in Supplementary Table S3. The most favorable score considering the three protomers was obtained for the 5γ site in the RBD subunit. While site 5γ is likely to be the most probable binding site, site 2 and site 3 are also probable candidates.

Considering site 5γ in protomer A, violacein was located in an environment defined by residues Phe338A, Tyr365A, Tyr369A, Ala372A, Ser373A, Phe374A, Asp405C, Glu406C, Arg408C, Gln409C, Thr415C, Gly416C, Lys417C (Figure 5A), finding a parallel-displaced π-stacking with Tyr365A, a T-shaped π-stacking with Tyr369A, hydrogen bonds with Tyr369A, Ser373A, and Thr415C, and a π-cation interaction with Arg408C (Figure 5B).

To further study the different interactions between violacein and the 5γ binding site in the protomer A, 50 nanoseconds of molecular dynamics (MD) simulation was carried out using GROMACS 2020.4 Molecular Dynamics engine (Abraham et al., 2015).

Figure 5C shows the root-mean-square deviation (RMSD) for violacein as a function of the simulation time. The average RMSD value was 0.645 Å with a standard deviation of 0.152 Å, indicating high ligand stability at the 5γ site. By exploring the interactions that stabilize the complex during the MD simulation through the measurement of the distances between violacein atoms and the key residues of the protein defined by docking (Supplementary Figures S1–S5), it can be concluded that the stabilization of violacein at the 5γ site appears to be mediated mainly by π-stacking and π-cation interactions, with a coordinated contribution from hydrogen bonding. The docking and MD procedure are described in the Supplementary Material together with a detailed analysis of the interactions observed in this study.

Finally, due to the novelty of this site, the SARS-CoV-2 Spike protein was further analyzed through the DoGSiteScorer web server, a grid-based method that uses a Difference of Gaussian filter to the binding pocket prediction, characterization, and druggability estimation (Volkamer et al., 2012). This analysis ranked the 5γ site in third place out of more than 100 potential sites found in the protein, with a druggability score of 0.85 (0.86 being the highest druggability score obtained).

2.9 Violacein production

The large-scale production of microbial pigments is seriously limited by the production-purification costs and regulations in dominant pharmaceutical countries. In 2018, the world market for organic dyes was valued at U$D 3.5 billion, with an estimated growth of about 37% by 2024 (Cassarini et al., 2021). The most representative studies on violacein production are briefly described below, including the use of recombinant microorganisms and the new trend of recycling waste in the frame of the circular economy.

Violacein production was initially studied in the 2000s. The first work on violacein production was conducted in *C. violaceum* and *Janthinobacterium lividum* in synthetic liquid media (Mendes et al., 2001; Nakamura et al., 2003). The main guidelines for the production of violacein from *C. violaceum* can be found in the websites of the American Type Culture Collection (ATCC, United States, https://www.atcc.org/products/12472), and the National Collection of Type Cultures (NCTC, United Kingdom, https://www.culturecollections.org.uk/products/bacteria/detail.jsp?collection=nctcandrefId=NCTC+9757).

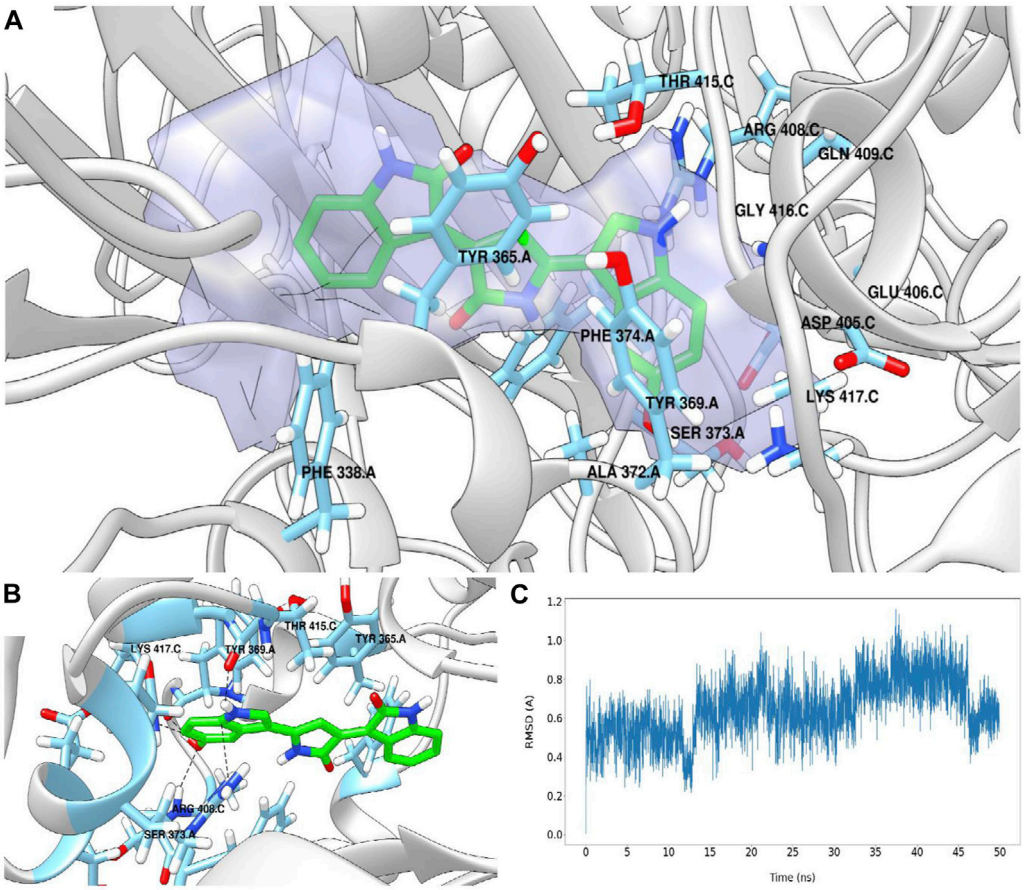

FIGURE 5
Binding site 5γ in protomer A predicted for violacein by molecular docking and characterized by the DoGSiteScorer web server **(A)**. Pose prediction for violacein. Some relevant interactions are shown with black dashed lines **(B)**. Violacein's RMSD (Å) vs. simulation time plot **(C)**.

In general, it is currently accepted that carbon sources play a crucial role in pigment synthesis; in particular, glycerol increases while glucose represses its production (Pantanella et al., 2007; Duran et al., 2021). These results indicate shared pathways for simple carbon sources, as well as the presence of catabolic repression by glucose. A systematic statistical study of violacein production by *C. violaceum* CCT 3496 cultured in a 50 mL flask containing glucose-rich media supplemented with tryptophan was conducted using a fractional factorial design followed by a central composite design. The violacein production increased from 7.5 g L^{-1} dry cell mass and 0.17 g L^{-1} crude violacein concentration to 21 g L^{-1} and 0.43 g L^{-1}, respectively (Mendes et al., 2001). The ratios of dry cell mass/crude violacein concentration were approximately 44 and 49 for initial and optimized production, respectively. These results indicate that coupled violacein production is related to bacterial cell mass.

The violacein producer *Duganella* sp. B2, isolated from a glacier in China, was statistically optimized using a two-level Placket-Burman, Box-Behnken, and surface response for biodye production. The experiments were carried out in 250 mL stirred flasks containing minimal saline medium and starch as a carbon source supplemented with tryptophan. The highest violacein concentration produced in the optimized medium was 1.62 g L-*1* after 32 h of culture (Wang et al., 2009).

The production of violacein from a wild-type isolated psychotropic bacterium RT102, close to *J. lividum,* cultivated in a 3-L fermenter containing rich media (*i.e.*, glucose, casein, and yeast extract) under physicochemical controlled parameters resulted in a maximum violacein concentration and productivity of 3.7 g L^{-1} and 0.12 g L^{-1} h-1 at 40 h culture (Nakamura et al., 2003).

Several efforts have been made to obtain recombinant bacterial strains that are capable of producing high concentrations of violacein. Most studies have focused on *E. coli* (Rodrigues et al., 2012; Rodrigues et al., 2013; Fang et al., 2015). The *E. coli* TOP10 strain transformed with the plasmids pJP1000 and pPSX-Vio + which are harboring the violacein operon *pBvioABCDE* from *C. violaceum* showed low levels of violacein production ranging from 0.006 to 0.025 g L^{-1}, which was comparable to the biodye production by *J. lividum* DSM 1522 under the same experimental conditions (Rodrigues et al., 2012). In another study by the same research group, different *E. coli* strains were transformed with plasmids and the integrated gene responsible for violacein production. The mutant *E. coli* dVio-1 and dVio-6 expressing the vioABCDE gene cluster accumulate 0.18 g L-1 and 320 mg L-1 of deoxyviolacein intracellularly, respectively. Integration of the vioD gene into the *E. coli* genome, *E. coli* Vio-4, produced 0.71 g L^{-1} of intracellular violacein by fed-batch fermentation (Rodrigues et al.,

2013). In future work, different recombinant *E. coli* BL21 strains were used as a chassis for the expression of violacein genes inserted in a plasmid associated with the enhanced production of tryptophan. Flask culture in glucose saline medium of the recombinant strain *E. coli* B2/pED + pVio produces 0.60 g L^{-1} violacein at a concentration of 48 h. Scale-up of violacein production in a fermenter with an optimized glucose medium showed a titer of 1.75 g L^{-1} associated with 36 mg L–1 h^{-1} biodye productivity (Fang et al., 2015).

In another microbial recombinant strategy, *Citrobacter freundii* containing the violacein gene cluster in a plasmid (i.e., pCom10vio) was cultured in a 2 L minimal salt medium supplemented with glycerol and tryptophan in a 5-L fermenter using a fed-batch technique. The optimized yield parameters were 3.34 g L-1 dry cell mass, maximum violacein concentration of 4.13 g L^{-1} with violacein productivity of 0.083 mg L^{-1} h^{-1} (Yang et al., 2011).

However, some issues must be taken into account for potential commercial applications of violacein in pharmaceutical applications because the high production and/or productivity of violacein in recombinant strains depends on the strain stability during scale-up and successive microbial cultures, violacein purification complexity (*i.e.*, steps and procedures) because the biodye is located intracellularly in most recombinant microorganisms, and the presence of violacein precursors and/or cometabolites (*i.e.*, protoviolaceinic acid, deoxyviolacein, etc.). Alternatively, the use of agricultural food waste can be an alternative to reduce violacein-producing costs as well as co-production with other metabolites of industrial interest. Pioneering investigations reported in the literature for violacein production were developed by Ahmad's research group at the University Technical of Malaysia. A wild-type strain of *C. violaceum* isolated from a waste plant has been extensively studied for the production of violacein in liquid media supplemented with local agricultural wastes and/or cheap substrates, such as brown sugar, molasses, solid pineapple waste, sugarcane bagasse, and commercial-rich media. Violacein production in the rich medium was very low or negligible. While the high biodye productions were 0.19 g L^{-1} and 0.82 g L^{-1} obtained in a medium containing 1% and 3% sugarcane bagasse supplemented with tryptophan, a pigment precursor, respectively (Ahmad et al., 2012).

Violacein production in *C. violaceum* UTM5 was studied by combining static and stirred growth conditions, and violacein production was between 0.17 g L^{-1} to 0.26 g L^{-1}. However, high production was observed when the microorganism was first cultivated under static conditions followed by stirring. After environmental optimized culture conditions, the microorganism produced 16.26 ± 0.44 g L^{-1} of violacein in a 50 L fermenter containing 45 L of the medium of filtered pineapple waste supplemented tryptophan at 30°C for 24 h (Aruldass et al., 2015).

Microparticulate wheat bran, a low-cost substrate with high lignocellulose content, was used to supplement the Luria-Bertani medium for violacein production using *C. vaccinii* in batch culture (Cassarini et al., 2021). The optimal violacein production was 0.208 g L^{-1} after 73 h of culture.

In a recent study, the simultaneous production of poly hydroxybutyrate (PHB) and violacein was reported (Kumar et al., 2021). The synthesis of PHB and violacein by wild-type *Iodobacter* sp. PCH194 was optimized in a 22-L fermenter containing glucose and tryptone in a saline medium. The maximum production of PHB was 11.0 ± 1.0 g L^{-1}, and a mixture of violacein (50%–60%) and deoxyviolacein (40%–50%) was obtained. The highest concentration of violacein was 1.5 g L^{-1} at 48 h of cultivation.

The main data on violacein production using different microorganisms, methodologies, and brief culture conditions are displayed in Supplementary Table S4. Another factor to be considered is related to the obtention of violacein from the culture media. Briefly, at the late stationary phase, the bacterial cells were centrifuged, and the violacein was extracted from the pellet with ethanol (Mendes et al., 2001).

Among the advantages of violacein production are the standard conditions of production, short times for production, the facile bacterial cultivation in fermenters, and scale up with traditional strains, the low nutritional requirements of the microorganism for the biodye production which could include wastes (*e.g.* with the advantage of the circular economy), easy recovery of the pigment and purification because of high hydrophobicity.

2.10 Development of violacein devices

Violacein attached to different materials has two main applications: as a filter barrier with antiviral activity or loaded into particulate systems for drug delivery (Khaksar et al., 2021; Fu et al., 2023).

Because the human coronavirus is mainly transmitted by aerosols caused by coughing, sneezing, breathing, or speaking, there is a need to establish a physical barrier for the dispersion of these aerosols. However, the infectivity over time of the coronavirus in a droplet of aerosol depends greatly on the surface where it is deposited, being able to remain active for several hours in some cases (Fu et al., 2023). This is why a functional material must not only retain droplets but also inactivate the virus.

Strategies have been developed to use violacein as a biodye in the development of functional fabrics, taking advantage of its antiviral properties. Three different methods for dyeing polyamide fabrics with violacein have been tested (Kanelli et al., 2018). Direct fermentation of *J. lividum* on the fabric, exposure of the fabric to the culture after fermentation, and exposure of the fabric to an acellular extract of the culture. Improved color retention and antimicrobial and biofilm inhibition activities were obtained using the first method (Kanelli et al., 2018). With the same goal of producing functional fabrics, Gao *et al.* (2019) developed a silk-based composite that takes advantage of the antimicrobial synergy between violacein and silver nanoparticles (Gao et al., 2019). Electrospinning techniques have also been tested to design fabrics based on violacein-containing synthetic polymers, such as Nylon-66 and Polyvinyl-alcohol/polyvinyl-pyrrolidone (Osman and Setu, 2018; Rosli and Setu, 2018). Also, by using electrospinning, Lee *et al.* (2022) have recently taken this concept further; developing prototype respiratory masks from polyacrylonitrile nanofibers with violacein, demonstrating aerosol retention from 0.8 to 3.4 μm (human-produced aerosols being between 0.74 and 2.12 μm), antimicrobial activity against *S. aureus* and antiviral activity against human influenza A and human coronavirus. Violacein also provides selective UV protection to the fabric (Lee et al., 2022). In particular, these violacein-loaded materials retain the inactivating activity of coronaviruses and influenza and could constitute products with real applications, such as surgical masks.

In another study, viscose fabric was incubated in the presence of violacein, followed by the incorporation of silver and titanium dioxide nanoparticles by sonication. The antimicrobial activity of the fabric containing violacein and nanoparticles showed a 3- to 6-log reduction in the growth of *S. aureus*, *B. cereus*, and *E. coli*, indicating the effectiveness of the fabric derivatized with violacein and nanoparticles (Khaksar et al., 2021).

Another potential application of violacein is to include biodye in 3D matrices for molecular delivery. In 2000, violacein was added to a *ß*-CD solution and precipitated. The violacein complexed in cyclodextrin showed the same biocide activity against *E. coli*, but the cytotoxicity in lung fibroblasts of Chinese hamster V-79 cell cultures was reduced, and lipid peroxidation was fully inhibited at 500 μM of the violacein-ß-cyclodextrin complex (). Later, a proof of concept was developed by the encapsulation of violacein in nanoparticles of poly-(D, L-lactic-co-glycolic acid) for assaying antimicrobial activity. PLGA nanoparticles with 116 and 139 nm diameter containing violacein efficiently inhibit five strains of *S. aureus*, including antibiotic-resistant (MRSA) strains, at micromolar concentrations three to five times lower than free violacein (Martins et al., 2011). In a subsequent study by Durán *et al.*, violacein was encapsulated in nanoparticles made of poly (ε-caprolactone) covered with chitosan to produce a positively charged device and increase mucoadhesiveness (Berni et al., 2013). Violacein nanoparticles of 201–320 nm were tested to prevent bovine mastitis against *S. aureus* MRSA with a low minimal inhibitory concentration compared to free biodye. Additionally, violacein encapsulation diminished ecotoxicity by approximately 10 to 5 times using Daphnia similis according to the OCDE guidelines.

Another strategy for violacein encapsulation is the use of Arabic gum as a matrix (Venil et al., 2015). Violacein microparticles can be used to provide color to foods, such as jellies and yogurts. Violacein encapsulated in Arabic gum was spray-dried and displayed high stability in the range of 25°C–60°C for 30 days.

Similarly, based on the low violacein solubility in aqueous media, the biodye was first suspended in a polyoxyethylene sorbitan monolaurate solution and showed high stability at room temperature for 6 days. Later, the violacein emulsion was successfully encapsulated in gelatin-pectin coacervate and tested against HCT-116 colon cancer (Rivero Berti et al., 2019). More recently, violacein was encapsulated in a nanostructured lipid carrier with an active release by lipase and 3D printed using a hydroxypropyl cellulose-chitosan matrix. The formulation was tested against the A549 and HCT-116 cancer cell lines (Rivero Berti et al., 2022).

Other strategies have been developed to increase the biocidal activity of violacein by using metallic nanoparticles. Silver nanoparticles with violacein adsorbed in the surface displayed high antibacterial activities against multiresistant bacterial strains of *P. aeruginosa, E. coli, and S. aureus*, also tested against *Aspergillus tamari, Aspergillus tubingensis*, and *Fusarium proliferatum*, and also algicidal activities against *Dictyosphaerium* sp. strains DHM1 and DHM 2, and *Pectinodesmus* sp. strain PHM 3. In all cases, the biocide activity of AgNPs capped with violacein against bacteria, fungi, and algae were enhanced by the presence of the biodye (Arif et al., 2017). Similar results were obtained for silk coated with AgNPs and violacein, as previously described (Gao et al., 2019).

A novel strategy for repositioning old antibiotics is to develop a combination of antibiotics and violacein. The authors developed a 1:1 individual mixture of 20 antibiotics with violacein and tested these mixtures against *Salmonella typhi*, *Vibrio cholerae*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. In most cases, the biocidal activity of violacein is synergistic and/or additive concerning the antibiotic (Subramaniam et al., 2014). This strategy offers several advantages. First, the antibiotic-violacein mixtures can increase the biocide activity against pathogens because of different molecular targets, decreasing the amount of the antibiotic concomitantly with a decrease in potential toxicities, and increasing the bioavailability of the components.

Additionally, the interaction of ten imidazole ionic liquids with violacein was analyzed. Imidazole ionic liquids and violacein form stable micellar solutions that are encapsulated in solid lipid nanoparticles. The anticancer activity of the violacein-imidazole ionic liquids formulation against A549, HeLa, and HCT116 cells was tested. These results indicated that the imidazole group of the ionic liquids associated with violacein displayed strong anticancer activity (Rivero Berti et al., 2020). Further computational studies have demonstrated the effectiveness of imidazole derivatives against SARS-CoV-2 (Belhassan et al., 2020). Data from both studies showed promising results for the use of violacein–imidazole ionic liquid formulations for SARS-CoVid-2 therapy.

Taking into consideration what is stated in this section, not only should the biological activity of violacein be considered, but also its ability to form functional materials. The late one is a relevant challenge that will result in a improve biocide and antiviral activities.

3 Conclusion

Many biological activities of violacein were extensively described in the literature. The previous focus was to use violacein in the treatment of different types of cancer. However, recently found violacein properties such as its effect on cellular membranes, and anti-inflammatory, antioxidant, and antiviral activities suggest that another application is possible. Other detailed studies of violacein activities revealed a wide spectrum of protease inhibition. Additionally, *in silico* studies through molecular docking confirmed the binding activities of violacein within the SARS-CoV-2 spike in several sites. However, in some cases, the antiviral titers of violacein are not enough to be considered as a potential virucide agent. These results could be attributed to two factors, first the presence of other molecules in the violacein extracts because of a lack of appropriate purification steps and quality controls, and second because of violacein low biodisponibility due to its very low solubility in physiological media. Besides, the production of violacein from different strains and procedures is in *the state of the art*, but it is essential to standardize pigment purification procedures. Also, the potential oral administration of violacein will require the development of novel strategies for molecular delivery systems to obtain more efficient biocide activities.

Author contributions

IR, writing, drawing, reviewing. MG, docking and molecular studies, writing. SR, docking and molecular studies, writing. GI, editing, reviewing. WF, editing, reviewing. AT, critical review, and text edition, discussion, and manuscript revision. ND, writing, text edition, and discussion. GC, organization, writing, editing, critical

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnano.2023.1186386/full#supplementary-material

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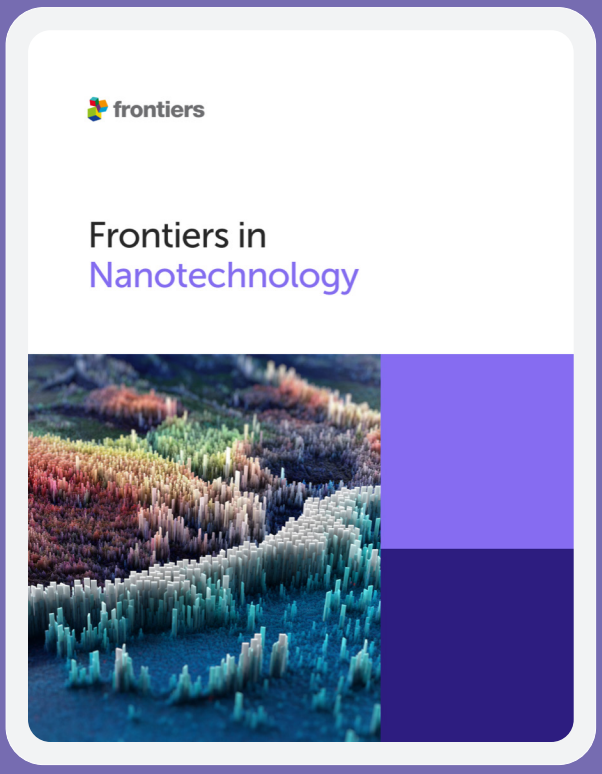

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