Nanoscience versus Viruses: The SARS-CoV-2 Case

Joanna Goscianska,* Ralph Freund, and Stefan Wuttke*

The outbreak of the coronavirus (SARS-CoV-2) pandemic, its rapid spread, and its fatal consequences clearly showed mankind the overwhelming power of nature, and the importance of interdisciplinary research to counter it. Herein, it will be pointed out what challenges arise in fighting nanosized viruses, which feature an outstanding ability to alter their structure and properties to adapt to infection processes, and why nanoscience is extremely powerful to address them. To learn from the past and to be best prepared for the future, this review highlights how the incredible potential of nanoscience can be tapped by mimicking nature’s viruses for the development of maximally efficient pharmaceuticals, e.g., COVID-19 vaccines, by designing preventive and protective equipment, e.g., face masks, as well as developing nanosensors for early detection of infections.

1. Introduction

Since time immemorial, humans have been facing various pandemics induced by different viruses, but the current health and global economy crisis caused by the spread of the coronavirus (SARS-CoV-2) has grounded humanity in unprecedented ways. The high population density and the massive increase in globalization and connectivity triggered an enormous speed of the spread so that people around the whole world were caught off guard by major disruptions in their lives. In these times of uncertainty about the course of events and the future, one thing is certain and is unmistakably felt by everyone: we must be better prepared for future virus attacks.

Over millions of years, nature has perfected the art of assembling the basic units of living beings, such as nucleic acids, proteins, macromolecular assemblies into larger, highly functional nanoobjects like viruses. Viruses, in their essence, can be regarded as masterpieces of nanocarriers that are programmed to deliver nucleic acids with unprecedented efficiency (Figure 1), and as a result, they can infect all types of life forms from plants over animals to humans. Having evolved over millions of years, viruses feature the remarkable ability to alter their structure and properties to adapt to the infection process, and are therefore ubiquitous in nature. Inspired by viruses’ intricate and highly efficient delivery systems, scientists have put immense effort into developing artificial nanocarriers with maximal therapeutic efficiency and minimal adverse effects (Figure 1).

To achieve this purpose, nanoscience has been aiming to reach a similar degree of sophistication by developing artificial nanosystems with a wide range of applications on the one hand and gaining an improved understanding of biological nanosystems on the other. The past two decades have definitely witnessed tremendous progress in different subfields of nanoscience and in pharmaceutical sciences toward this goal: i) chemists have learned to synthesize increasingly complex nanoparticles through “bottom-up” assembly; ii) physicists and engineers have developed methods, mostly using “top-down” techniques, to fabricate nanoscale materials; and iii) biophysicists and biochemists have had increasing success in manipulating and controlling the interactions at the bio–nanointerface. Based on these important achievements, a wide range of different nanocarrier material classes could be established, including liposomes, polymers, micelles, dendrimers, inorganic nanoparticles made of iron oxide, quantum dots, noble metals, carbon or silica, as well as hybrid organic–inorganic materials such as metal–organic frameworks.[1–10] It is worth noting that the European Medicines Agency (EMA), as well as the U.S. Food and Drug Administration have already approved some of these nanocarriers as suitable for addressing challenges in the development of systemic drug delivery platforms.[3,11–15] Also, the administered vaccines in the current SARS-CoV-2 pandemic, such as Pfizer-BioNTech and Moderna, are based on lipid nanocarriers that facilitate the delivery of mRNA into cells and exhibit an antiviral efficiency as high as 95% and 94.1%, respectively.[16–18]

Such successful examples highlight the great promise of nanoscience to combat viruses and encourage further research toward the development of nanocarrier platforms.
Fostered by the cutting-edge, highly interdisciplinary nature of viruses and nanoscience as well as the immense current interest in both topics (Figure 2), this review aims to transfer nanoscience into the context of viruses using SARS-CoV-2 as a model example that led to a new research direction. Specifically, we will highlight how nanoscience can solve various timely problems in the treatment, prevention, and diagnosis of viruses like SARS-CoV-2: i) synthesis of novel drugs and vaccines based on nanocarriers with enhanced efficiency and reduced side-effects for the treatment of the current pandemic and the prevention of future virus attacks; ii) design of protective equipment based on nanoparticles, such as medical face masks and outfits; and iii) development of nanobiosensors for early detection of infections in a maximally efficient way.

2. Development of Nano-Based Vaccines

2.1. Types of Vaccines

To date, massive vaccination has been one of the most effective means of preventing and spreading infectious diseases by stimulating antibody and T cell responses to induce protective long-term immune memory.[19–21] The use of vaccines leads to a reduction of mortality and disease complications. In the process of vaccine design, the first priority is to define the antigen that affects the body’s immune response, the adjuvant—the co-administered auxiliary substance responsible for prolonging, enhancing, and modulating the immune response against the antigen, and the delivery strategy.[22] The combination of these data together with the bioinformatic predictions and epitope (antigenic determinant) mapping is extremely important for the modern vaccine design. It should be emphasized that many types of vaccines are either currently in use or under development, which include inactivated and live-attenuated vaccines (first generation), subunit, conjugated, and recombinant vaccines (second generation), as well as DNA and RNA vaccines (third generation) (Figure 3).[23]

The live-attenuated vaccines comprise weakened forms of the original pathogen. Thus, they induce strong cellular and antibody responses, giving long-term immunity after administration of usually one or two doses. It is easier to develop live-attenuated vaccines with viruses than with bacteria due to the fact that viruses have fewer genes. The greatest limitation of these vaccines is the necessity of refrigeration to maintain potency and the ability of pathogen reversion to the original virulent forms. It is also worth mentioning that the live-attenuated vaccines cannot be taken by immunocompromised individuals because they produce actual disease manifestations.[21,23,24]

By contrast, inactivated vaccines do not pose a risk to individuals with weakened immune systems, however, they may not...
provide sufficient protection for people with B cell or combined immunodeficiency. They are created by the inactivation or killing of a pathogenic agent via its exposure to radiation, chemical, or thermal treatment, resulting in a safety and stability increase (Figure 3). It is proved that these vaccines elicit weaker immune responses, therefore, additional doses are indispensable to retain immunity. Moreover, the possibility of the pathogen’s virulence features recovery is of high concern.

An alternative to live-attenuated and inactivated vaccines is the second-generation vaccines that rely on subunit elements, recombinant or synthetic proteins, nonprotein antigens, and expressed bacterial immunogen or viruses (Figure 3). Subunit vaccines contain the only epitopes (protein–peptides or polysaccharides naturally occurring in pathogenic structure), stimulating the immune system, thus the likelihood of adverse reactions is lower. In the case of conjugate vaccines, proteins are used to enhance the immunogenicity of polysaccharide antigens. These vaccines have helped to reduce the disease incidence, especially in infants. Recombinant vaccines are fabricated using recombinant DNA technology, which enables gene sequencing of the main pathogenic antigen, stimulating an immune response. In order to induce immunogenicity, a small DNA piece from the virus or bacterium is retrieved and incorporated into the manufacturing cells. It was found that in comparison to other vaccines, recombinant vaccines are safer, particularly for immunocompromised patients. The main limitation of the second-generation vaccines is the weak immunogenicity of the antigens, posing the need for an adjuvant introduction to enhance immune response.

At last, the third-generation vaccines known as genetic ones are based on a plasmid administration, comprising a gene encoding intentional antigen that is expressed in the cells by means of a specific promoter, causing the immune response (Figure 3). DNA vaccines have a lot of advantages but they are still under development for cancer therapies, allergies, autoimmune and infectious diseases. Besides the stability at various temperatures, they are also cost-efficient, safe, facile in manufacturing, and capable of stimulating humoral, mucosal, and cellular immunity. These types of vaccines have some downsides such as the potentiality to be used only for protein antigens, inability to get to target sites, and untimely degradation of the antigens leading to a weakened immune response. Hence, the efforts of the scientific community are currently focused on the development of new vaccines using the benefits of nanotechnology. Along with the contemporary trend of research aimed at understanding the immune cell interactions at the sub-micrometer level, nanotechnology will play an increasing role in future immunology discoveries.

2.2. The Impact of Nanomaterials on the Improvement of Vaccine Responses

The nanotechnology platforms offer great opportunities in the process of designing new vaccines, being the milestones for medicine and biotechnology. Recently, nanovaccines have drawn huge attention of scientists, who are constantly working on optimizing nanoformulations to efficiently load and safely deliver genetic material (Figure 4). The great achievement reached so far is that both nanoparticles and viruses (including SARS-CoV-2) can act on the same length scale. Moreover, natural and synthesized nanomaterials can simulate the structural features of various viruses. In contrast to drug release systems that can be designed to target a specific cell type, an
immunogenic nanomaterial-based vaccine must interact with the different cell types, e.g., professional antigen-presenting cells, B cells, T cells, macrophages, and neutrophils. This interplay can take place in many tissues over extended periods of time, making the rational design of nanomaterial vaccines a complex endeavor. One thing is certain, in the case of this type of vaccine, the morphology, and size of nanoparticles, their durability in vivo, the antigen physical orientation, adjuvant specific codelivery, as well as complement activation can be precisely controlled. The listed parameters affect the vaccine pathway to the different lymphoid tissues which determine the quality and potency of the immune response.

When an antigen is encapsulated within nanocarriers or conjugated (bound) to the surface of nanoparticles, its persistence increases at the injection site, in lymphoid tissues, and in circulation. This, in turn, may enhance the antigen immunogenicity which is highly desirable. To achieve this goal, various nanotechnological solutions have been proposed. For instance, it was established that the nanoparticles consisting of the multilamellar lipid vesicles transporting a recombinant malaria antigen, both entrapped in the aqueous core and attached to the lipid bilayer surfaces, increased the stability and the strength of humoral immune B cell responses in a synergistic manner. Demento et al. investigated the impact of nanoparticle-mediated antigen delivery on the long-term stability and extent of the vaccine response. It was demonstrated that extended-release of ovalbumin—an antigen model encapsulated within poly(lactic-co-glycolic acid) nanoparticles can improve immunogenicity. Additionally, by controlling the nanoparticle degradation rate, the ovalbumin release can be further prolonged. Thus, it can be suggested that the selection of the appropriate vehicle ensures gradual, lasting availability of the antigen, which is a key factor facilitating a long-term memory T cell response and body recovery after infections with various pathogens.

Reaching a controlled and programmable delivery of nanoparticle-based vaccines to the B cells, T cells, follicular dendritic cells and subcapsular sinus macrophages is an extremely important aspect to consider in the vaccine design process. In recent years, many nanosystems have been developed to act as carriers for antigens. These include liposomes, polymeric nanoparticles, virosomes, inorganic nanoparticles, emulsions, and immune-stimulating complexes which will be comprehensively discussed in the next section.

The nanoparticle systems that are contained in the new generation vaccines have the ability to precisely control, enhance, and optimize the density and orientation of the antigen. The introduction of the antigens onto the nanomaterial surface via physical adsorption is based on the weak electrostatic or noncovalent hydrophobic interactions. This can result in rapid detachment of the antigen from the nanoparticle in vivo. The nanocarriers such as chitosan, dextran sulfate polymeric
nanoparticles, gold nanoparticles, and carbon nanotubes have a higher affinity to the amphoteric antigens. Therefore, the antigen release process can be designed by controlling the properties of the biological environment like pH, temperature, or ionic strength.\textsuperscript{[34,37–39]} In the case of encapsulation and chemical conjugation (Figure 5), the interplay between the antigen and the nanoparticle is significantly stronger. In the first process, the antigens and the nanoparticle precursors are mixed during the synthesis. Hence, only when the nanoparticle is degraded in vivo or inside the cell, antigens can be released.\textsuperscript{[19]} Contrarily, in chemical conjugation, the antigen is chemically crosslinked to the nanoparticle surface. In this form, it is uptaken by the cell and then released inside the cell.\textsuperscript{[40]}

The antigens are generally delivered to lymph nodes (LNs) by the antigen-presenting cells (APCs), which gather antigens at the injection site and transport them to the LNs to activate T and B cells and produce adaptive and memory immune response for long-term immunity. However, drainage to the LNs may also occur, which is mainly dependent on the size of the employed nanoparticles. It has been demonstrated that the nanoparticles with sizes in the range of 10–50 nm tend to drain to the LNs,\textsuperscript{[41]} while those with a diameter below 6 nm drain to the vasculature.\textsuperscript{[42]} At the same time, other parameters such as flexibility, shape, and surface charge also influence the drainage of the nanoparticles to the LNs.\textsuperscript{[43]} In order to circumvent the need to take into account all these factors, the best solution could be an intranodal vaccine injection.\textsuperscript{[44–46]} In this case, the accumulation and degradation of the vaccine at the site of injection are not observed and it is possible to deliver more cargo to LNs. Clinical trials involving this approach have proven to be very promising.\textsuperscript{[47–49]} Jewell and co-workers\textsuperscript{[46,50]} studied direct LN injection of biodegradable polymer nanoparticles loaded with antigen and adjuvant. The results confirmed potently enhanced CD8$^+$ T cell response within 7 days after a single injection of vaccine with controlled-release components in small animal models. Strong, long-lasting humoral immunity was also generated. Compared to traditional peripheral injection routes, it was possible to significantly minimize the dose of the vaccine and improve its efficacy. However, it should be mentioned that the anatomical positions and size of the LNs can limit the translation of this vaccination method.

The mucosal immune response is also essential for respiratory, sexual, and oral transmission of viruses.\textsuperscript{[51]} In this regard, special attention has been paid to the delivery of nanoparticles through the mucosa.\textsuperscript{[19]} This process depends on the pore size and surface features of the mucosal barriers. Cervicovaginal mucus has the mean pore diameters of 340 nm\textsuperscript{[52]} whereas respiratory mucus of up to 200 nm, therefore, only the nanoparticles with sizes smaller than these values can penetrate through the barriers.\textsuperscript{[53]} Especially, the cationic nanoparticles exhibit high mucoadhesion, so they can be retained in mucus and interact strongly with mucosal immune cells.

It is worth emphasizing that nano-based vaccines can also ameliorate the action of adjuvants and reduce negative side effects, e.g., toxicity and inflammation. Application of the nanoparticles to deliver adjuvants may contribute to their dose reduction through controlled release near or even inside APCs.\textsuperscript{[54]} In particular, for subunit vaccines, adjuvants are crucial elements to shape immunological memory. It was shown that mice immunization with lipid vesicles incorporating Plasmodium vivax circumsporozoite antigen and delivery in combination with the monophosphoryl lipid A adjuvant stimulated the high-titer antibody responses against VMP001 that lasted for more than 1 year at tenfold lower doses than in the case of traditional adjuvants.\textsuperscript{[29]} Friede et al.\textsuperscript{[55]} indicated that liposomes containing nontoxic monophosphoryl lipid A adjuvant can be used as carriers, causing a long-lasting IgG response against peptides. This approach can be helpful for the development of synthetic vaccines as it eliminates a need to employ protein carriers and conventional adjuvants. Finally, some nanoparticles exhibit intrinsic adjuvant activity, resulting from their composition and/or structure, which may facilitate the formulation of novel vaccines.\textsuperscript{[37]}

The use of stimuli-responsive nanocarriers in the design of new vaccines has also become groundbreaking. These types of smart nanomaterials undergo specific physicochemical changes under the influence of interaction with internal and external stimuli.\textsuperscript{[56]} The cellular organelles including endosomes and lysosomes reveal sharp pH differences in the intracellular microenvironment.\textsuperscript{[57]} A pH-sensitive nanocarrier can facilitate the liberation of vaccine components at specific target sites and enable controlled antigen presentation by APCs to elicit desired immune responses. It was found that biomimetic micelles composed of the dendritic cell (DC) membrane, histidine-modified stearic acid-grafted chitosan, and ovalbumin exhibit pH-dependent antigen release resulting in their effective escape from DC lysosomes.\textsuperscript{[58]} Liu et al.\textsuperscript{[59]} fabricated pH-responsive poly(\(\mathrm{d},\mathrm{L}\)-lactic-co-glycolic acid) (PLGA) nanoparticles characterized by thin shells and large inner space containing ovalbumin antigen and \(\text{NH}_4\text{HCO}_3\) acting as a promoter of antigen release in DCs. \(\text{NH}_3\) and \(\text{CO}_2\) react with hydrogen ions in DC endosomes and lysosomes (pH \(\approx 5.0\) and 6.5) to generate \(\text{NH}_3\) and \(\text{CO}_2\) causing destruction of nanoparticles and effective release of antigen to the cytoplasm (Figure 6). The immunization of mice with pH-responsive PLGA NPs elicited greater lymphocyte activation, more antigen-specific CD8$^+$ T cells, and enhanced antigen-specific IgG antibodies, demonstrating cellular immunity. The pH-responsive PLGA nanoparticles have great potential to be used as effective vaccine delivery and adjuvant systems for the therapy of virus infections.

A combination of antigen and adjuvant co-delivery with pH-responsive endosomal membrane disruption may provide an even stronger immunostimulatory effect. Wilson et al.\textsuperscript{[60]} obtained micellar endosomolytic nanoparticles for delivery of immunostimulatory CpG oligodeoxynucleotide adjuvants and ovalbumin antigen to enhance cellular and humoral immunity. Mice immunization with subcutaneous injections of the nanoparticle formulations significantly augmented CD8$^+$ T cell response compared to administration of the free agents.

The temperature change is another intrinsic stimulus that can affect the release of the antigen from the nanocarrier. thermo-responsive materials, which mainly include hydrogel-based polymers are characterized by the unique property of altering solvation state depending on temperature. They can transform from free-flowing liquids at room temperature to gels at physiological conditions without needing chemical or other environmental stimulations.\textsuperscript{[56,61]} The modified thermo-responsive polymers such as poloxamer 407 and chitosan–methyl
cellulose formulations have the ability to create an immobilized biomaterial depot for controlled antigen release, loaded by simple mixing at room temperature, and inducing an immunological response. It was found that the chitosan–methyl cellulose sol–gels sustained liberation of ovalbumin antigen up to at least 14 days after administration and stimulated both cellular and humoral responses in mice.\(^{[62]}\) Future smart vaccination strategies could also exploit thermo-responsive and immunomodulatory properties of hydrogel-based polymers to prolong the release of vaccine adjuvants. It should be emphasized that the sustained vaccine efflux could initiate the innate and adaptive immune response for long-term immunological memory.\(^{[56,63]}\)

2.3. Nanoparticles Used in Vaccine Development

Nanoparticle-based vaccines provide an excellent alternative to conventional vaccines due to numerous advantages, such as tunable sizes and shapes, tailorable surface properties, controllable antigen release kinetics, and improved stability. As previously mentioned, many nanoparticles have been developed for vaccine platforms. Depending on the components creating their structure, they can be divided into organic (e.g., natural and synthetic polymer-based nanoparticles, liposomes, virus-like particles) and inorganic (e.g., gold, mesoporous silica, carbon, and calcium phosphate nanoparticles) (Figure 7). Biocompatibility, nontoxicity, and biodegradability are characteristic features of the majority of organic nanomaterials, whereas inorganic nanoparticles exhibit small particle size, good stability, enhanced permeability, ability to functionalize the external/internal surface, and high sorption capacity (Table 1).\(^{[64,65]}\) In this section, we will describe examples of nanoparticles used in the vaccine design process with particular emphasis on their physicochemical properties, functions, and interaction with antigens.

2.3.1. Polymer-Based Nanoparticles

Natural polymers, namely chitosan, alginate, inulin, and pululran are of great interest due to the possibility of their application to prepare nanoparticle adjuvants and vaccine delivery vehicles.\(^{[34,39,99]}\) They are advantageous primarily due to their biocompatibility, biodegradability, mucoadhesive properties, facile modification, as well as size and shape tunability.\(^{[66,67]}\) Loading of listed polymeric nanoparticles with antigens relies on ionotropic gelation and self-assembly of the polyelectrolytes without employing organic solvents or high shear stress which allows the immunogenicity of the antigens to be preserved.\(^{[66,100]}\) In the case of the development of a vaccine against HIV, antigens can be chemically conjugated to chitosan to provide more stable bonds and prevent untimely antigen release.\(^{[101]}\) The cationic nature of chitosan facilitates encapsulation of anionic antigens,\(^{[101,102]}\) nucleic acids\(^{[103]}\) and other

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Figure 6. Schematic illustration of the composition and structure of pH-responsive PLGA nanoparticles and mechanism of their action. Reproduced with permission.\(^{[59]}\) Copyright 2015, American Chemical Society.
negatively charged biopolymers.\cite{104} Thanks to mucoadhesiveness, chitosan nanoparticles have been used for the delivery through the mucosa,\cite{102–107} causing enhanced dendritic cell uptake and supporting the opening of the tight epithelial cell junctions in the mucosal tissues for the antigen paracellular transport.\cite{108} Chitosan nanoparticles have been also utilized in the preparation of DNA vaccines because they can complex with DNA and protect it from nuclease degradation,\cite{109–112} as well as in Newcastle disease vaccines,\cite{113} and hepatitis B virus (HBV) vaccines.\cite{114,115}

Water-soluble sodium alginate is commonly used in biomedical applications, especially for the protection of encapsulated antigens against hydrochloric acid and protease present in the stomach, after oral administration, and also for controlled release of the incorporated antigens.\cite{116,117} The attenuated *Androctonus australis hector* insect toxins were encapsulated in calcium-alginate nanoparticles to design vaccines against scorpion envenomation.\cite{100} Mannose-functionalized alginate nanoparticles conjugated by Schiff base bond with an ovalbumin were developed for cancer immunotherapy.\cite{116} They promoted antigen uptake of bone marrow dendritic cells and antigen cytosolic release which confirms their great potential as a nanovaccine.

Inulin composed mainly of fructose units and terminal glucose, after crystalization in the delta polymorphic form, becomes immunologically active.\cite{118} It can be used as an adjuvant. It was demonstrated that nanoparticle adjuvant Advax obtained from inulin enhanced immune response in vaccine influenza\cite{118} and against hepatitis B\cite{119} with low reactogenicity and no adverse effects.

Many various synthetic polymers are commonly used to obtain nanoparticles for biomedical applications including poly(lactic acid) (PLA), poly(\(\beta\),\(\delta\)-lactide-co-glycolide) (PLG), poly(lactic-co-glycolic acid) (PLGA), poly(\(\gamma\)glutamic acid) (\(\gamma\) PGA), poly(ethylene imine) (PEI), poly(\(\varepsilon\)-caprolactone) (PCL), poly(anhydride) (PAN), and poly(N,N-cystaminebis(acrylamide)-co-4-amino-1-butanol) (pABOL).\cite{30,66,120–123} Polyester-based nanoparticles exhibiting excellent biodegradability and bio-compatibility have been developed to efficiently carry antigens in vaccines. Their size, surface chemistry, and hydrophobicity can be precisely controlled during synthesis providing the possibility to modulate the release of incorporated antigens influencing immune responses. One of the most important advantages of polyester-based particulate vaccines is the ability to administer via different routes, from intranasal, oral, intradermal, and intramuscular to subcutaneous.\cite{123} To generate nanoparticles from PLGA and PLA, the double emulsion method is usually employed that facilitates antigen encapsulation within the polymer matrix.\cite{124} Moreover, this method permits the coencapsulation of antigens with immune-stimulating adjuvants or excipients.\cite{125,126} Alternative approaches to load antigens rely on physical adsorption or chemical conjugation to the particle surfaces.

So far, PLGA has been applied as carriers for antigens derived from different pathogens, such as Plasmodium vivax with monophosphoryl lipid A as adjuvant,\cite{127} HBV, Bacillus anthracis,\cite{129} and model antigens such as ovalbumin and tetanus toxoid.\cite{30,130} In turn, \(\gamma\)PGA nanoparticles are used for encapsulation of hydrophobic antigen.\cite{131,132} PEI and its functionalized derivatives have been designed mainly for the delivery of nucleic acid vaccines, including antigen-encoding DNA,\cite{113} messenger RNA (mRNA),\cite{134} and self-amplifying replicon RNA (repRNA).\cite{135} Complexes of mannosylated PEI with plasmid DNA that encode fifteen different HIV antigens have been used as therapeutic nanoparticle vaccines for HIV.\cite{136,137} These PEI nanoparticle vaccines named DermaVir are currently under phase III clinical trial. Other cationic polymers such as pABOL are also used for effective delivery of the nucleic acids, e.g., self-amplifying RNA (saRNA) encoding firefly luciferase in mice.\cite{138} Sub-100 nm PCL nanoparticles were loaded with hepatitis B surface antigen for the development of a single-dose oral vaccine. It was demonstrated that mice vaccinated with the PCL nanoparticles generated significant antigen-specific antibody.
titers for both systemic and mucosal immunity.\textsuperscript{[139]} In turn, protein antigens from culture filtrates of \textit{Mycobacterium avium} subsp. \textit{paratuberculosis} lysates were incorporated into PAN nanoparticles with a diameter of 200 nm and then administered subcutaneously to mice. Antigen-specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells were identified after performed vaccination.\textsuperscript{[140]}

2.3.2. Liposomes

Liposomes are spherical, artificial vesicles formed from cholesterol and natural nontoxic phospholipids.\textsuperscript{[69]} Due to their biocompatibility, small size, and hydrophobic/hydrophilic nature, they can be used as delivery systems for the combination of multiple antigens and adjuvant molecules.\textsuperscript{[70,71,141,142]} The main benefit of vaccines relying on liposomes is plasticity and versatility. The selection of the bilayer composition and methods of obtaining liposomes enables the achievement of the desired features such as charge, size, size distribution, rigidity or fluidity, entrapment, and location of antigens or adjuvants. The use of unsaturated phosphatidylcholine species from natural sources (e.g., egg or soybean phosphatidylcholine) leads to form bilayers of high permeability and lower physical stability, while the saturated phospholipids with long acyl chains (e.g., dipalmitoylphosphatidylcholine) are suitable for creating rigid, impermeable bilayer structures.\textsuperscript{[69]} It was found that liposomal subunit vaccines are distinguished by safety, low reactogenicity, and biodegradability. Reactogenicity concerns rare immune responses, causing symptoms like fever, allergies, or pain at the injection site. This kind of vaccine is composed of antigen(s) (protein, lipid, lipopeptide, etc.) incorporated into the lipid bilayer or the liposome core. The liposome acts as an adjuvant, which enhances the immune responses of the vaccine, improving its effectiveness.\textsuperscript{[141]} The incorporation of antigen can be realized with various methods, such as encapsulation, surface adsorption, covalent lipid conjugation (before or after vesicle creation), noncovalent surface attachment (by antibody–epitopes interactions), or electrostatic interactions (with an oppositely charged lipid).\textsuperscript{[143]} Brunel et al.\textsuperscript{[144]} demonstrated the efficiency of recombinant hepatitis B surface antigen based on DC–cholesterol (Chol) liposomes or aluminum hydroxide adjuvants in a subcutaneous vaccine model. In comparison to conventional vaccines containing aluminum hydroxide and showing very weak immunogenicity, DC–Chol liposomes elicited controlled Th1 and Th2 immune responses. Mahor et al.\textsuperscript{[145]} developed novel cationic transfersomes for topical DNA vaccine delivery. Cationic transfersomes consisting of cationic lipid DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane) and sodium deoxycholate as constitutive lipids were loaded with plasmid DNA encoding hepatitis B surface antigen (HBsAg). It was found that DNA-loaded cationic transfersomes induced higher anti-HBsAg antibody titer and cytokines level than

| Type of nanoparticles | Size | Shape | Characteristics | Ref. |
|-----------------------|------|-------|----------------|------|
| Polymers              | 10–2000 nm | Spheres, truncated spheres, hemispheres, capsules | Nontoxicity, Biocompatibility, Biodegradability | [34,39,66,67] |
| Liposomes             | 25–1000 nm | Spheres | Nontoxicity, Biocompatibility | [34,39,68–71] |
| Immunostimulating complexes (ISCOMS) | 40 nm | Cage-like particles | Cytotoxicity-mediated immune responses | [34,39,68,72–74] |
| Virus-like particles (VLPs) | 20–800 nm | Icosahedra, rods, spheres | Self-assembled nanoparticles containing biocompatible recombinant proteins | [34,39,68,75–77] |
| Nanoemulsions         | 20–200 nm | Spheres | Nontoxicity, Nonirritant | [39,78–80] |
| Gold nanoparticles    | 2–150 nm | Spheres, cubes, rods | Immunotoxicity, Non-biodegradability, More potent immune response | [39,68,72,81,82] |
| Carbon nanotubes (CNTs) | Diameter: 0.8–2 nm Length: 100–1000 nm | Tubes | Nontoxicity, Good biocompatibility, High stability, Nonimmunogenicity | [39,68,83–88] |
| Silica nanoparticles  | 50–500 nm | Spheres, hollow spheres, rods | Size-dependent toxicity, Biodegradability in the cell membranes, Tunable mesoporous structure | [36,39,96,97,68,89–95] |
| Calcium phosphate (CaP) nanoparticles | 50–100 nm | Spheres | Low cytotoxicity, Excellent biocompatibility, Biodegradability | [39,68,98] |
was observed for unmodified DNA. Maleimide-functionalized liposomes can be prepared as interbilayer-crosslinked multilamellar vesicles utilizing cation-driven fusion and crosslinking, which provide a gradual release of the entrapped antigen. It is worth noting that some liposome systems, namely, Inflexal V and Epaxal have been developed and approved for human use.

### 2.3.3. Immunostimulating Complexes

Immunostimulating complexes (ISCOMs) are particles with a cage-like structure and size of about 40 nm, combining a multimeric presentation of antigen with a built-in adjuvant. They consist of saponin adjuvant Quil A, cholesterol, protein antigen, and phospholipids. ISCOMs containing antigens are characterized by very high stability hence their structure remains intact after lyophilization, freeze-thawing, and extended storage at 4 °C. These spherical particles can be considered as vehicles for antigen and adjuvant aiming at delivering their combination to APCs and the lymphatic system. Importantly, ISCOMs with incorporated appropriate antigens can elicit mucosal immunity and evoke secretory IgA or cytotoxic T cell responses after oral and intranasal administration. It was found that the experimental vaccine formulations based on ISCOMs enable effective prevention and spreading of infectious diseases caused by various pathogens such as viruses, retroviruses, parasites, and bacteria. So far, antigens derived from influenza, herpes simplex virus, HIV-1 and Newcastle disease have been used to formulate ISCOMs.

#### 2.3.4. Virus-Like Particles

Virus-like particles (VLPs) are nonreplicating virions devoid of infectious nucleic acid, which are formed as a result of the self-assembly of biocompatible capsid proteins. VLPs with the appropriate size and repetitive structural order may constitute the optimal nanovaccine platform because they resemble virus structures precisely designed to induce strong immune responses even without adjuvant. Moreover, it was reported that these systems are characterized by good stability and fewer side effects. The first vaccine based on VLPs for hepatitis B virus was launched in 1986. Further, high safety VLP vaccines for healthy people against human papillomavirus and hepatitis E were approved worldwide in 2006 and 2011, respectively. A large number of VLP vaccines against, e.g., influenza virus, parvo virus are currently under clinical trials or preclinical evaluation, therefore an increase in the number of approved vaccines can be expected in the near future. Commercialized VLPs have been manufactured in various expression systems, such as Escherichia coli, baculovirus-insect cell, yeast, and Chinese hamster ovarian mammalian cell. The structural complexity and immunogenicity of the desired VLPs primarily determine the choice of expression host for VLP production, but the cost should also be taken into account. It is anticipated that the use of plants as biofactory or cell-free procedures may be the future of VLPs subset production. VLPs can serve as delivery systems in which additional non-VLP related antigen is introduced onto their surface. Such platforms also take all the benefits of VLPs, including optimized particle size and molecular structure to combat infectious diseases.

#### 2.3.5. Nanoemulsions

Nanoemulsions are another type of nanoparticles that can be successfully applied as adjuvants in vaccine delivery. They constitute a thermodynamically stable isotropic system prepared by mixing two immiscible phases (water and oil) and employing suitable surfactants. The droplet sizes of nanoemulsions are usually in the range of 20–200 nm. They are characterized by a much higher surface area and free energy compared to macroemulsions. In addition, they are nontoxic and nonirritant. Nanoemulsions are able to transport antigens inside their core. An oil-in-water nanoemulsion, MF59, is widely used as a safe and potent vaccine adjuvant introduced into licensed influenza vaccine in more than 20 countries. It was reported that MF59 influences the immunogenicity of flu vaccines in the elderly, and also in chronically ill adults. Moreover, the use of this type of nanoemulsion allows reducing the dose of the vaccine taken against pandemic influenza. Two other adjuvants rendering water-in-oil emulsions, Montanide ISA51 and Montanide ISA720 have also been developed. The first one is a mixture of mineral oil and surfactant from the mannan monooleate family. In the latter case, mineral oil is replaced with nonmineral oil of vegetable origin. Montanide ISA51 and Montanide ISA720 have been examined in the clinical trial of vaccines for AIDS, malaria, and cancer immunotherapy. The formulations were well-tolerated but elicited transient local reactions like pain, discomfort, swelling. At present, Montanide ISA201 and 206 adjuvants are utilized for the preparation of foot-and-mouth disease vaccine in many South American and Asian countries due to their facile injectability and no side effects.

#### 2.3.6. Inorganic Nanoparticles

Gold nanoparticles of 2–150 nm in size are widely used as carriers in vaccine delivery. They can be easily synthesized by various methods allowing precise control of their diameter and shapes by changing such parameters as temperature, time, type of the reducing agent, and solvent. Both the shape and size of Au nanoparticles have an impact on the immune response of the host cells. The studies of Niikura et al. on Au nanoparticles coated with West Nile virus envelope protein demonstrated that gold nanorods (40 × 10 nm) are more efficient in macrophage and dendritic cell uptake than spherical (20 and 40 nm in diameter) or cubic (40 × 40 × 40 nm) Au nanoparticles. However, the 40 nm spherical Au nanoparticles elicited the highest level of West Nile virus envelope protein-specific antibodies. Therefore, it can be concluded that antibody production is not determined by uptake efficacy. Au nanorod-treated cells produced significant levels of interleukin-1β (IL-1β) and interleukin-18 (IL-18), indicating that they activated inflamasome-dependent cytokine secretion. Meanwhile, both...
spherical and cubic Au nanoparticles elicited inflammatory cytokine production (Figure 8).\cite{171}

Interestingly, it was proved that spherical Au nanoparticles of a diameter ranging from 8 to 17 nm conjugated with synthetic foot-and-mouth disease virus peptide showed the highest antibody response in comparison to the synthetic peptide conjugate.\cite{172} Au nanoparticles can be utilized as vehicles for antigens originating from the influenza virus\cite{173} and as an adjuvant for the human immunodeficiency virus DNA vaccine.\cite{174} It should be mentioned that functionalization of Au nanoparticles with carbohydrate conjugates is another approach used in order to improve the immunogenicity of vaccine. Safari et al.\cite{175} prepared Au nanoparticles conjugated to synthetic tetrasaccharide epitopes from Streptococcus pneumoniae, which induced specific anti-Pn14PS IgG antibodies, causing cytokine production of TNF-α, IL-2, and IL-5 in mice spleen and activation of memory T-cell.

Carbon-based nanomaterials, such as carbon nanotubes (CNTs), carbon spheres, and graphene have gained great attention in the scientific community corresponding to their unique physicochemical features advantageous for many biomedical-related fields.\cite{176-180} Particularly, CNTs are the exciting claimants to be used in nanovaccine formulations as scaffolds for carrying the specific antigen and facilitating its presentation to the immune system.\cite{83-86} CNT carriers possessing diameters in the range of 0.8–2 nm and lengths of 100–1000 nm are relatively inert, stable, nonimmunogenic, and nontoxic.\cite{85,87,88} Their distinctive structures enable introducing the combination of a number of antigens onto their surfaces simultaneously. Another advantage is the ability to penetrate into cells, including dendritic cells, which are essential for stimulating efficient immune responses.\cite{85,181} Bacterial, protozal, and viral antigens beside CpG adjuvants can be conjugated to CNTs. The first reports of the use of CNTs as a scaffold for vaccine development concerned the covalent attachment of the peptide from the foot-and-mouth disease virus, which induces neutralizing antibody responses. It was found that the structure of the peptide’s epitope in its antigenic form is preserved after attachment to CNTs. The peptide linked to CNTs elicited virus-specific immune responses in mice. Moreover, no antibodies against the CNT structure were generated indicating the great potential of CNTs as carriers for antigens.\cite{182,183} In another work, Plasmodium vivax apical membrane antigen-1 peptide was conjugated to multiwalled CNTs to treat malaria. The specific immune response was directed toward the peptide rather than CNTs, revealing that carbon vehicles are not intrinsically immunogenic.\cite{184} Meng et al.\cite{185} developed a therapeutic cancer vaccine based on a combination of tumor lysate protein with single-walled CNTs in mouse model hepatoma. The tumor lysate protein–CNTs conjugate improved noticeably the rate of cure and enhanced the cellular antitumor immune reaction in comparison to the lysate itself. Mocan and Iancu\cite{186} studied the therapeutic potential of multiwalled CNTs combined with embryonic stem cells as novel vaccine nanobiosystems. They demonstrated that concurrent immunization with embryonic stem cells and multiwalled CNTs elicit significant antitumor

![Figure 8. TEM images of gold nanoparticles. A) Sphere20 (20 nm in diameter), B) Sphere40 (40 nm in diameter), C) Cube, and D) Rod. Scale bar represents 40 nm. Mechanism of gold nanoparticle action and West Nile virus envelope protein-specific IgG ELISA end point titers in mice immunized twice at 3 week intervals with 100 ng of Au nanoparticles. Reproduced with permission.\cite{171} Copyright 2013, American Chemical Society.](image-url)
responses and enhance tumor rejection in mice with subcutaneous inoculation of malignant colon cells.

Silica-based nanoparticles are one of the most interesting inorganic candidates for biomedical applications such as nanovaccination,[36,91,92] drug delivery,[187] biosensing,[188] bone tissue engineering,[189] cancer therapeutics, and diagnostics.[190] They possess many beneficial properties, among which the most important are high specific surface area and pore volume,[191,192] biocompatibility,[193,194] colloidal and thermal stability,[193] as well as biodegradability in the cell membranes.[95] Moreover, silica nanoparticles with sizes in the range of 50–200 nm have tunable structural parameters and morphology determining the cellular uptake and internalization rates.[96,97,194] A large amount of silanol groups on the silica surface allows the introduction of various organic/inorganic functionality in order to improve chemical stability and interaction with cells.[195–198] All these features are of great importance for effective encapsulation, high loading capacity, and controlled release of various biomolecules.[199] So far, three types of silica nanoparticles (S1, S2, and SBA-15) differing in sizes (430 nm, 130 nm, 1–2 μm) and pore characteristics, were loaded with bovine serum albumin (BSA) model protein to design a vaccine delivery platform. It was established that the immune response was remarkably determined by the uptake and antigen release profile. The structure of the silica was also of great importance. The rate of protein release varied with the size of the silica vehicle pores. The large pores enhanced the interaction between BSA and nanoparticles, stimulating antigen gradual release.[200] Slowing et al.[200] suggested that the reason for the slow antigen release from silica nanoparticles may be connected to the high stability and rigidity of a framework, creating a barrier against antigen degradation in the stomach and digestive tract. The hollow mesoporous silica nanoparticles (HMSNs) have been utilized as antigen delivery carriers for Porcine circovirus type-2 (PCV2) ORF2 protein. It was demonstrated that HMSNs improve both humoral and cell-mediated immune responses. The percentage of CD4+ and CD8+ T cells in the mice immunized with HMSN/GST-ORF2-E was higher than that observed for mice immunized using the only HMSNs at 4th and 6th-week of research.[201] Mahony et al.[92] studied immunization by the ovalbumin model protein antigen employing MCM-41 mesoporous silica nanoparticles functionalized with amine groups. Functionalization of material led to a 2.5-fold increase in ovalbumin binding capacity compared to the nonmodified sample. Moreover, amine-modified nanoparticles acted as self-adjuvants and induced immune responses at reduced antigen doses in vivo. No local or systemic negative symptoms in animals after injection with silica nanoparticles were observed. The presented examples show that silica nanoparticles have a great potential to be used as vehicles stimulating antigen-controlled release in future vaccine formulations.

Calcium phosphate (CaP) nanoparticles prepared by mixing calcium chloride, dibasic sodium phosphate, and sodium citrate under specific conditions have sizes ranging from 50 to 100 nm.[202–204] Sodium citrate is used as a stabilizing agent and a regulator of crystal growth. Due to the chemical similarity to biological hard tissues, CaP nanoparticles show excellent biocompatibility and low cytotoxicity. They can act as central binding sites for nucleic acids, antigenic proteins, and cells, protecting these biomolecules from external stressors and permitting delivery to endosomes/lysosomes.[99] After releasing encapsulated biomolecules, CaP nanoparticles are decomposed due to the change in pH of the environment from neutral into acidic during cellular uptake, which makes them ideal vehicles. It was found that these nanoparticles are valuable and easily biodegradable adjuvants for DNA vaccines and mucosal immunity.[85,202,203] Moreover, CaP nanoparticles constitute an improved alternative to alum adjuvants in mice immunized with viral proteins.[203]

The studies utilizing different nanoparticles as delivery systems and/or adjuvants in vaccine design are summarized in Table 2.

2.4. Nanotechnological Strategies for COVID-19 Vaccine Design

At the outset, it should be noted that vaccines for infectious diseases had never been produced at such a rapid rate as in the case of the COVID-19 vaccines. Moreover, no vaccine existed for preventing infections caused by coronaviruses in humans. The vaccine design process is extremely long and expensive. It usually includes many years of research on different candidates from which the most effective and the safest one is selected. Due to the high costs and failure rates, the vaccine development process follows a linear sequence of steps, such as preclinical vaccine design, efficacy and safety studies, clinical trials—phases I–III, licensure, with breaks for in-depth data analysis and manufacturing process control. Rapid vaccine design requires a new pandemic paradigm of multiple steps in parallel, hence there is an increased financial risk (Figure 9).[206–209] As soon as China announced in December 2019 that a novel coronavirus was the cause of the Wuhan outbreak, international efforts were focused on the accelerated development of modern vaccine formulations. With the potential for further financial support, vaccine production began when the SARS-CoV-2 genetic sequences were released by the Chinese Center for Disease Control and Prevention.[210–212] SARS-CoV-2 is an enveloped single-stranded ribonucleic acid ((+ssRNA) virus with glycoprotein spikes that protrude from its external membrane surface creating a characteristic “corona.” The coronavirus particle integrity is maintained by four major structural proteins: i) spike (S) protein enabling the attachment of the virus to the host cells; ii) envelope (E) protein playing a structural role and assisting in assembly as well as budding; iii) the abundant membrane (M) protein preserving the integrity of viral particle membrane; and iv) nucleocapsid (N) protein mostly binding to the RNA of SARS-CoV-2 and supporting the formation of the nucleocapsid (Figure 10).

In particular, S protein is a splendid target for the design of vaccines, but it is worth highlighting that antigen optimization is necessary to ensure an adequate immune response.[213] The S protein of SARS-CoV-2 is cleaved into two subunits: S1 and S2. S1 includes the receptor-binding domain (RBD) and is responsible for attachment to the cell. S2 is composed of N and C heptad repeats, a potential fusion peptide, and a transmembrane domain. It facilitates the membrane fusion and entry into the cell.[20,212–215] By combining information on the structure of SARS-CoV-2 and the knowledge gained during the
development of SARS/MERS vaccine candidates, the full length S protein, S1, RBD, and S2 subunit derivatives were designed to contain the main target epitopes to elicit neutralizing antibodies. From the vaccine technology development perspective, this is an inspiring period in which the novel nano-based approaches can make a great clinical impact for the first time.

Although currently many different COVID-19 vaccines are approved or under development, in this section, we will focus only on nanotechnology-based ones (Table 3).

The mRNA vaccines have many advantages as pandemic-protection platforms, given their flexibility and efficiency in immunogen design and production. As mentioned earlier, for these types of vaccines, lipid nanoparticles are often selected as delivery systems. mRNA complexed with positively charged lipids is characterized by higher stability and resistance to RNase-mediated degradation. They form self-assembled virus-sized particles ensuring the possibility to be administered via various routes. After endocytosis, the lipid nanoparticles release their genetic cargo in the cytosol, where the mRNA is translated into antigenic proteins, stimulating the immune system to produce neutralizing antibodies. The current limitation of this type of vaccine formulation is their long-term storage requiring low

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**Table 2. Nanoparticle-based vehicles and adjuvants used in vaccine development.**

| Nanomaterial                              | Immunogen                              | Disease                                    | Function                          | Ref.     |
|-------------------------------------------|-----------------------------------------|--------------------------------------------|------------------------------------|----------|
| Alginate-coated chitosan nanoparticles    | Hepatitis A surface antigen             | Hepatitis A                                | Adjuvant/delivery system           | [99]     |
| Calcium-alginate nanoparticles            | *Androctonus australis* hector venom    | Scorpion envenomation                      | Delivery system                    | [100]    |
| Chitosan nanoparticles                    | Newcastle disease virus                 | Newcastle disease                          | Delivery system                    | [113]    |
| Chitosan nanoparticles                    | Hepatitis B surface antigen             | Hepatitis B                                | Delivery system                    | [114]    |
| Chitosan nanoparticles                    | Hepatitis B surface antigen             | Hepatitis B                                | Delivery system                    | [115]    |
| Inulin                                    | Inactivated virus from H1N1, H3N2, and B strains | Influenza                                  | Adjuvant                           | [118]    |
| Inulin                                    | Hepatitis B surface antigen             | Hepatitis B                                | Adjuvant                           | [119]    |
| PLGA                                      | Hepatitis B surface antigen             | Hepatitis B                                | Delivery system                    | [128]    |
| PCL nanoparticles                         | Hepatitis B surface antigen             | Hepatitis B                                | Delivery system                    | [139]    |
| Liposomes                                 | Self-replicating RNA genome             | Chikungunya fever                          | Delivery system                    | [142]    |
| Cationic lipid DC-Chol                    | Hepatitis B surface antigen             | Hepatitis B                                | Adjuvant                           | [144]    |
| Cationic transfersomes                    | Plasmid DNA encoding hepatitis B surface antigen | Hepatitis B                                | Delivery system                    | [145]    |
| ISCOMs                                    | Envelope proteins of Newcastle disease virus | Newcastle disease                          | Delivery system                    | [148]    |
| ISCOMs                                    | Influenza virus                         | Influenza                                  | Adjuvant/delivery system           | [149]    |
| ISCOMs                                    | Herpes simplex virus type 2 (HSV-2) antigen | Herpes                                    | Delivery system                    | [150]    |
| ISCOMs                                    | Chimeric peptide containing V3 loop and transmembrane sequence of gp41 with two glycine motifs | HIV-1                                    | Delivery system                    | [151]    |
| VLPs                                      | RSV fusion protein                      | Respiratory syncytial virus infection      | Delivery system                    | [153]    |
| VLPs                                      | HIV-1 envelope protein                  | HIV-1                                      | Delivery system                    | [154]    |
| VLPs                                      | Viral capsid formed by the ORF2 protein | Hepatitis E                                | Delivery system                    | [158]    |
| Oil-in-water nanoemulsion, MF59           | Antigen influenza viral strains         | Influenza                                  | Adjuvant                           | [163–165]|
| Water-in-oil nanoemulsion, Montanide ISA51 | C-terminal 19-kDa fragment of the *P. yoelii* merozoite surface protein 1 | Malaria                                    | Adjuvant                           | [167]    |
| Water-in-oil-in-water nanoemulsion, Montanide ISA201 | Antigen from virus strain of FMDV O/IND/R2/73 | Foot-and-mouth disease                    | Adjuvant                           | [169]    |
| Au nanoparticles                          | WNV (West Nile Virus) envelope protein  | West Nile fever                            | Delivery system                    | [171]    |
| Au nanoparticles                          | Virus peptide                           | Foot-and-mouth disease                     | Delivery system                    | [172]    |
| Au nanoparticles                          | Matrix 2 protein (M2e) of influenza A virus | Influenza A                               | Delivery system                    | [173]    |
| Au nanorods                               | HIV-1 Env plasmid DNA                  | HIV-1                                      | Adjuvant/delivery system           | [174]    |
| Au nanoparticles                          | Spike protein of SARS-CoV               | Severe acute respiratory syndrome-related coronavirus infection | Adjuvant/delivery system | [205]    |
| CNTs                                      | Virus peptide                           | Foot-and-mouth disease                     | Delivery system                    | [182,183]|
| CNTs                                      | *Plasmodium vivax* apical membrane antigen-1 peptide | Malaria                                   | Delivery system                    | [184]    |
| CaP nanoparticles                         | Viral glycoproteins                     | Herpes simplex virus type 2 and Epstein-Barr virus infections | Adjuvant | [202,203]|
| CaP nanoparticles                         | Plasmid DNA                             | Foot-and-mouth disease                     | Adjuvant/delivery system           | [204]    |

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*Adv. Funct. Mater. 2022, 32, 2107826*
temperatures and in turn leading to logistical difficulties in distribution.\[217\]

In 2020, BioNTech (Biopharmaceutical New Technologies) German company in conjunction with Pfizer American multinational pharmaceutical corporation started a coordinated program aimed at comparing four RNA-based COVID-19 vaccine candidates in clinical studies performed both in Germany (BNT162-01) and in the United States (C4591001). It was important to choose one vaccine candidate and the dose level for further international safety and efficacy trials. Bearing in mind preliminary clinical-trial results in Germany,\[218\] two lipids nanoparticle-formulated\[219\] nucleoside-modified RNA vaccines against COVID-19 were assessed in the U.S.\[220\] The first one, BNT162b1, encodes the SARS-CoV-2 receptor-binding domain, trimerized by adding a T4 fibrin foldon domain to enhance its immunogenicity. In turn, the second candidate, BNT162b2, encodes the SARS-CoV-2 full-length spike, modified by two proline mutations to lock it in the prefusion conformation and simulate the virus which interacts with the induced virus-neutralizing antibodies. In these vaccine formulations, lipid nanoparticles were used as mRNA carriers, protecting the nucleic acid from degradation and allowing delivery of the mRNA into cells.\[221\] BNT162b2 was proved to have a better safety profile than the other vaccine candidates,\[222\] and a two-dose regimen provided 95% protection against COVID-19 in individuals 16 years of age or older.\[16\] Moreover, Pfizer and

Figure 9. Difference between the traditional and accelerated development of vaccines for viral diseases. The outbreak paradigm is associated with a high financial risk of stages occurring simultaneously. For the SARS-CoV-2 vaccines, the knowledge gained from the initial design of vaccines against SARS-CoV and MERS-CoV was taken into account. Therefore, it was possible to omit the discovery phase.

Figure 10. The structure and morphology of SARS-CoV-2. Reproduced with permission.\[216\] Copyright 2020, American Chemical Society.
BioNTech declared that the vaccine is safe and 100% effective for 12–15 years aged adolescents, which was established based on the phase III trial data. Among all participants of the trial, only 6% reported adverse events after first and second doses.\[223,224\]

Another lipid nanoparticle-encapsulated mRNA vaccine named mRNA-1273 was designed by Moderna American pharmaceutical and biotechnology company and the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases, within the National Institutes of Health.\[210,225\] This mRNA-1273 vaccine encodes the SARS-CoV-2 spike (S) glycoprotein stabilized in its prefusion conformation. It should be noted that the S glycoprotein mediates host cell attachment and is necessary for viral entry.\[226\] In the case of mRNA-1273, PEGylated lipid nanoparticles act as delivery vehicles. The mRNA-1273 vaccine demonstrated 94.1% efficacy at preventing COVID-19, very similar to that observed for the BNT162b2 vaccine (95%) developed by Pfizer and BioNTech.\[17\] Moreover, after its administration, only transient local and systemic reactions were identified.\[17,227\]

Preliminary results did not demonstrate any obvious negative signals among pregnant women after administration of mRNA COVID-19 vaccines: BNT162b2 and mRNA-1273. Further long-term monitoring of the large number of women vaccinated during the first trimester of pregnancy should be conducted to determine possible effects on pregnancy and infants.\[228\]

Japanese pharmaceutical company Daiichi Sankyo in collaboration with the University of Tokyo’s Institute of Medical Science developed also a gene (mRNA) vaccine for COVID-19 called DS-5670a with the use of nucleic acid delivery technology based on lipid nanoparticle structures. Clinical trials of this vaccine are expected to start in 2021 (NCT04821674).\[229\]

Lu et al.\[230\] developed three mRNA vaccine candidates for COVID-19 named RQ3011-RBD, RQ3012-Spike, and RQ3013-VLP encoding different antigen forms in vaccinated hosts. The first one encodes the RBD of the spike (S) glycoprotein modified by two proline mutations and encapsulated in LNPs. The second vaccine RQ3012-Spike encodes the full-length wild-type S. In turn, RQ3013-VLP is utilized to incorporate mRNAs. The efficiency of encapsulation

| Developer-Maker | Name | Type | Characteristics of vaccine | Status |
|-----------------|------|------|----------------------------|--------|
| BioNTech (U.S.)/Pfizer (U.S.)/Fosun Pharma (China) | BNT162b2 | mRNA vaccine | Vaccine encoding the SARS-CoV-2 full-length spike glycoprotein modified by two proline mutations and encapsulated in LNPs | Approved Phase IV* (NCT04760132, NCT04780569, NCT04775069) |
| Moderna/National Institutes of Health (U.S.) | mRNA-1273 | mRNA vaccine | Vaccine encoding prefusion stabilized SARS-CoV-2 spike glycoprotein; PEGylated LNPs act as delivery vehicles | Approved Phase IV* (NCT04760132, NCT04792367) |
| Daiichi Sankyo/University of Tokyo, Institute of Medical Science (Japan) | DS-5670a | mRNA vaccine | Gene (mRNA) vaccine with the use of nucleic acid delivery technology based on LNPs | Phase I/II (NCT04821674) |
| Sanofi Pasteur/Translate Bio (U.S.) | MRT5500 | mRNA vaccine | 2P/GSAS S mRNA encapsulated in an ionizable LNP formulation | Phase I/II (NCT04798027) |
| Fudan University/Shanghai JiaoTong University/Novavax Biopharma (China) | RQ3013-VLP | mRNA vaccine | LNPs loaded with mRNAs cocktail encoding three structural proteins: S, M, and E to create SARS-CoV-2 VLPs with natural proteins: S, M, and E to create SARS-CoV-2 VLPs | Preclinical |
| Fudan University/Shanghai JiaoTong University/Novavax Biopharma (China) | RQ3011-RBD | mRNA vaccine | LNPs loaded with mRNAs encoding the receptor-binding domain (RBD) of the spike glycoprotein | Preclinical |
| CanSino Biologics (China)/Precision NanoSystems (Canada) | mRNA-LNP | mRNA vaccine | Vaccine candidate comprising of mRNA and lipid nanoparticles obtained with the NanoAssembler manufacturing technology | Preclinical |
| Inovio Pharmaceuticals (U.S.) | INO-4800 | DNA vaccine | DNA plasmid encoding spike glycoprotein; intradermal administration followed by electroporation with the use of CELLECTRA 2000 device | Phase II/III (NCT04642638) |
| Novavax (U.S.) | SARS-CoV-2 rS (NVX-CoV2373) | Subunit vaccine | Vaccine formulation containing modified SARS-CoV-2 spike glycoprotein combined with Matrix-M adjuvant | Phase III (NCT04611802) |
| IMV Inc. (Canada) | DPX-COVID-19 | Subunit vaccine | Peptide epitopes from SARS-CoV-2 spike glycoprotein encapsulated in LNPs | Preclinical |
| Medicago/GSK (Canada) | CoVLP | Recombinant vaccine | Recombinant spike glycoprotein expressed as VLPs administered together with GSK’s adjuvant (AS03) | Phase II/III (NCT04636697) |
was higher than 98% in the case of all three vaccine candidates. It was demonstrated that although the RQ3012-Spike and RQ3013-VLP contained the same amount of S mRNA, only the latter induced humoral and T cell immune responses. Moreover, it also produced the highest titers of neutralizing antibodies. The least promising vaccine candidate turned out to be RQ3011-RBD, which did not induce sufficient immunity in mice. However, this does not exclude the use of RBD as an antigen in future mRNA vaccines, but some improvements must be made to increase immunogenicity.\textsuperscript{[239]}

To date, no DNA vaccine for COVID-19 has been licensed. However, it should be mentioned that this kind of vaccine besides antibody and CD4\textsuperscript{+} T cell responses induces also CD8\textsuperscript{+} cytotoxic T cell responses which play a crucial role in neutralization of virus infection. Smith et al.\textsuperscript{[240]} presented the preliminary results on immunogenicity of the synthetic DNA-based vaccine candidate, INO-4800, targeting SARS-CoV-2 S protein. Scientists used their previous experience with the development of vaccines against MERS-CoV (INO-4700) and Zika virus (GLS-5700) that are currently under clinical trials. It was found that INO-4800 is a promising vaccine candidate because it elicits cellular and humoral host immune responses observed within several days after a single immunization in mice and guinea pigs. At present, DNA vaccine leader—Inovio Pharmaceuticals biotechnology company is assessing INO-4800 vaccine against SARS-CoV-2 in the three mid-stage trials in China, collaborating with Advaccine Biopharmaceuticals Suzhou Co., Ltd., in a mid-to-late stage trial in the U.S. and one in South Korea.\textsuperscript{[241,242]}

On 11 March 2021, Novavax biotechnology company from Maryland has published interim phase III trial data for a subunit coronavirus vaccine named NVX-CoV2373. Interestingly, this vaccine acts differently compared to other already approved vaccines and it has demonstrated to exhibit final efficacy of 96.4% against mild, moderate, and severe disease caused by the original SARS-CoV-2 strain in the United Kingdom. Moreover, it is also efficient in 55.4% of the HIV-negative trial participants in South Africa where the majority of strains are B.1.351.\textsuperscript{[243]} The data from the 2019nCoV-302 study, conducted with participants aged 18 to 84 in UK, showed that a two-dose regimen of the NVX-CoV2373 vaccine is safe and provides 89.7% protection against the B.1.1.7 coronavirus strain.\textsuperscript{[244]} This variant is more transmissible and causes higher mortality than previous strains. The Novavax protein-based vaccine was engineered from a modified SARS-CoV-2 spike gene inserted at the beginning into baculovirus, which then infected the culture of Sf9 moth cells. The infected cells produced spike proteins that are harvested and assembled into nanoparticles. Although the nanoparticles simulated the structure of the coronavirus, they can neither replicate nor induce COVID-19 disease.\textsuperscript{[245]} The NVX-CoV2373 vaccine formulation contains Matrix-M patented adjuvant composed of the nanoparticles of 40 nm in diameter based on saponin with cholesterol and phospholipid, enhancing the immune response and stimulating high levels of neutralizing antibodies.\textsuperscript{[246–248]} Tian et al.\textsuperscript{[239]} proved that in mice and baboons, low dose of NVX-CoV2373 vaccine with adjuvant induce high titer anti-S IgG and functional antibodies blocking hACE2 receptor binding and eradicate virus infection.

Subunit vaccines can take the form of VLPs described in detail in Section 2.3.4. They are considered a powerful candidate to prevent spreading the SARS-CoV-2 pandemic. Highly ordered and stable VLP vaccines are manufactured using recombinant expression. An example is the plant-derived COVID-19 vaccine developed by Medicago, a biopharmaceutical company from Quebec City, and GlaxoSmithKline (GSK). The proposed approach utilizes living plants as bioreactors to produce recombinant spike (S) glycoprotein expressed as VLPs which are then administered together with GSK’s adjuvant.\textsuperscript{[239]} VLPs simulate the native structure of the virus, so they can be readily recognized by the immune system. On 16 March 2021, Medicago and GSK companies have announced that the phase III clinical trials with healthy individuals aged 18–65 years were started. They will continue thereafter with elderly adults (65y+) and adults with comorbidities. All participants of the study will take two doses of vaccine 21 days apart.\textsuperscript{[240]} A similar approach for the development of several COVID-19 vaccine candidates was applied by the researchers from the University of Bristol and spin-out firm Imophoron. The production of the vaccines relies on the insertion of harmless bits of the viral surface proteins into the ADDomer platform creating synthetic VLPs that mimic coronavirus. The high specificity of the spike (S) part essential for cell entry decreases the risk of negative side effects. The new highly adaptable platforms for vaccines are extremely stable without refrigeration and enable easy production on a large scale. They will be tested in vivo in a preclinical program.\textsuperscript{[241,242]}

### 3. The Role of Nanomedicine in Antiviral Therapies

Currently, large-scale global research efforts have focused primarily on developing effective COVID-19 vaccines that will significantly reduce morbidity, disease complications, and mortality. However, after over 200 years of vaccine research, and observing hundreds of thousands of deaths every year as a result of different infectious diseases (where vaccines are available), the need for constantly improving therapies for virus-infected patients is obvious. In order to be better prepared for virus attacks in the future, research work is underway to repurpose existing drugs and evolve novel antiviral therapies to halt progressive illness that can lead to respiratory failure and consequently to death.

The development of antiviral therapies, as for vaccines, requires many years of research before they can be widely used due to the series of regulatory steps necessary to establish the efficacy and safety of drugs. On the other hand, the highly specific viral targets may change because SARS-CoV-2 continues to mutate causing drug resistance. This phenomenon has been commonly observed in attempts to treat other viral infections. In recent years, the interest in novel antiviral compounds has grown because they may be less susceptible to resistance and have great potential for use against a wide range of viruses, including new mutations and variants. Both artificial intelligence and other computation tools are largely used to support the development of drugs and delivery systems. Nanotechnology provides excellent opportunities to combat viruses both outside and inside the host, which is proven by numerous solutions that have already been applied.\textsuperscript{[245,243–245]} A vast number of...
promising nanocarrier-based antiviral treatments are currently under investigation to address the limitations of conventional therapy related to heterogeneous drug biodistribution and intracellular trafficking—cell-specific targeting and molecular transport to specific organelles. In order to facilitate the development and clinical translation of all the most perspective solutions, in 2000 the U.S. National Science and Technology Council launched the National Nanotechnology Initiative and defined the greatest challenges regarding the application of nanoparticles in diagnostics and the treatment of infectious and civilization diseases.

3.1. Nanocarrier-Based Antiviral Drug Delivery Systems

Clercq and Li reported that 90 antiviral drugs are approved to treat viral infections, but most of them only work against certain types of virus. Hence, when a completely new virus emerges, its pharmacological control and spread are limited. SARS-CoV-2 mainly attacks the respiratory tract (upper airways, lungs), however other organs (e.g., gut, kidney) and systems (e.g., vasculature) can be also impacted. It is believed that the expression of ACE2 influences which organs are subject to infection. In the case of SARS-CoV-2, attempts have been made to treat patients with antivirals such as lopinavir, ritonavir, remdesivir, chloroquine, hydroxychloroquine, and their various combination.

Lopinavir and ritonavir belonging to protease inhibitors have been successfully used to treat HIV infection and they have improved the outcomes of MERS-CoV and SARS-CoV patients. The recent studies reported that administration of lopinavir/ritonavir (Kaletra, AbbVie, U.S.) significantly reduced β-CoV titers of the COVID-19 patient in Korea. However, it was also revealed that lopinavir/ritonavir treatment neither reduced mortality nor diminished throat viral DNA in patients with serious symptoms of COVID-19. Future trials may confirm or deny the medicinal benefit resulting from the use of lopinavir and ritonavir.

Ribavirin is a purine nucleoside analog exhibiting broad-spectrum antiviral activity which is primarily applied for the treatment of respiratory syncytial virus infections and also in combination with interferon α for the therapy of chronic hepatitis C. Ribavirin has also shown antiviral activity against SARS-CoV and SARS-CoV-2 in vitro. So far, clinical studies performed with ribavirin have resulted in ambiguous outcomes. The reason for this may be that the concentration of ribavirin required to attain inhibition was difficult to achieve in clinical settings. Despite this, when used as a part of combination therapy with the HIV-1 protease inhibitor—lopinavir, the concentration of ribavirin required to inhibit SARS-CoV was reduced to 6.25 μg mL⁻¹. Hence, ribavirin may still be successfully used as a part of combination COVID-19 therapies with other antiviral drugs, interferons, or HIV-protease inhibitors. However, its use can cause hemolytic anemia.

Another example of a drug used in COVID-19 therapy is remdesivir (GS-5734), an inhibitor of the viral RNA-dependent RNA polymerase. It exhibits antiviral activity in vitro against the Ebola virus, Marburg virus, Paramyxoviridae (e.g., Nipah virus, parainfluenza type 3 virus, Hendra virus, measles, mumps viruses), Pneumoviridae (e.g., respiratory syncytial virus), SARS-CoV-1 and the Middle East respiratory syndrome (MERS-CoV). The clinical trial of intravenous remdesivir in adults has demonstrated potent antiviral activity for SARS-CoV-2, significantly shortening the recovery time of hospitalized patients with lower respiratory tract infection. The most common side effects connected with the administration of this antiviral drug in people with COVID-19 are nausea, liver inflammation, low blood pressure, and sweating.

The drugs representing the inhibitors of viral entry are chloroquine and hydroxychloroquine. Chloroquine is a derivative of quinine that has been widely used for decades to treat malaria. In addition to its antimalarial properties, this drug has shown broad-spectrum antiviral effects against a diverse range of viruses, such as the dengue virus, Zika virus, chikungunya virus, and influenza viruses. It also proved to be an effective antiviral drug against SARS-CoV in vitro when introduced prior to or after the establishment of infection. Recently, it displayed similar, potent antiviral effects against SARS-CoV-2. In addition to its direct antiviral activity, chloroquine also modulates the host immune system. Preliminary results from two clinical trials have demonstrated the efficacy of chloroquine in reducing SARS-CoV-2 viral load in the majority of patients. However, it often causes serious side effects, including vomiting, heart problems, headache, vision changes, and muscle weakness. Liu et al. performed the comparative analysis between hydroxychloroquine and chloroquine against SARS-CoV-2 infected patients. The results show that hydroxychloroquine is less toxic and more effective in inhibiting SARS-CoV-2 infection. Moreover, in a multi-hospital assessment, when controlling COVID-19 risk factors, treatment with hydroxychloroquine alone and in combination with azithromycin was associated with a reduction in COVID-19 associated mortality.

This short presentation of antiviral examples showed that their administration is very often connected with negative side effects caused by their accumulation in nontarget organs. Furthermore, these drugs are very often characterized by poor aqueous solubility and low bioavailability. The presented limitations of the drugs used in COVID-19 and other viral therapies can be solved by biocompatible, safe nanocarrier-based drug delivery platforms that will improve their efficiency and bioavailability, reduce toxicity, and maintain the viral spread suppression in plasma. They should also help to keep subtherapeutic concentrations of antivirals in reservoir sites and prevent premature release and degradation of drugs, as well as avoiding renal and hepatic clearance, nanocarriers can provide an improvement in their half-life. Thus, they are designed for controlled biodistribution and release of antiviral drug molecules at the target site within a specified time. In general, pharmacokinetic properties (i.e., absorption, distribution, metabolism, excretion) are inherent characteristics of drug molecules that cannot be changed without derivatization. The nanoparticles, which are small enough to circulate in the bloodstream and penetrate into the deep lung, are attractive materials for utilization as drug containers since their pharmacokinetic features can be tuned without affecting the activity of loaded drugs. Key parameters such as size, shape, charge, hydrophobicity, and specific
interactions with target cells determine the biodistribution of nanocarriers. However, it should be mentioned that even under normal physiological conditions, effective drug-loaded nanoparticle biodistribution and delivery can be difficult to achieve because they encounter many physical and biological barriers, e.g., shear forces, rapid clearance, and protein adsorption, limiting their fraction reaching target sites.[287,288] These barriers differ not only across specific disease states, but also on a patient-to-patient basis and may appear at the systemic, microenvironmental, and cellular levels. Their deep understanding is essential for designing optimal drug delivery nanosystems. The appropriate route of administration is also significant because it can improve nanoparticle biodistribution, affect their stability and properties as well as alter antiviral drug efficacy in vivo (Figure 11).[289] For example, in the case of PLGA nanoparticles intravenously injected, their accumulation was observed mainly in the liver and spleen. On the other hand, if they are subcutaneously or intranodal injected, they are more likely to accumulate in local lymph nodes.[291] Such alternative methods of nanoparticle administration allow them to reach the lymphatic system prior to systemic circulation.[292] Oral administration is the most common and easiest way to deliver drugs. If encapsulated in nanoparticles, they encounter numerous barriers in the gastrointestinal tract.[293] Crossing the endothelium by nanoparticles relied on passive diffusion is restricted by concentration gradients and P-glycoproteins excreting drugs from the vasculature into the intestinal lumen. In turn, for nanoparticle-based strategies based on endocytosis and subsequent exocytosis, the size is a key factor determining the ability to cross the alimentary canal. Polymeric nanoparticles with a high surface area are beneficial for oral administration due to the enhanced interactions with the gastrointestinal tract.[294] The optimal size for transcytosis of nanoparticles was found to be around 100 nm.[294–296] Another, in recent years more and more often considered route of nanoparticle administration, is inhalation. Its advantage is the omission of the systemic circulation and hepatic first-pass metabolism before delivery of nanoparticles directly to the lungs and lymph node.[289] This administration route could especially be very effective for viral infections. However, in addition to improved transport of nanoparticles to lung tissue, they may face obstacles in the form of mucus and pulmonary surfactants acting as physical barriers to lung delivery which significantly change across patients and pathologies.[295,296] Due to the different deposition rates and heterogeneous distribution of polymeric nanoparticles in the lungs by intratracheal instillation, intratracheal spraying, and intranasal instillation, there is a need to develop validated methods of nanoparticle pulmonary administration.[299]

To date, many drug nanocarriers have been developed that enable their rapid or controlled release. Currently, synthesis strategies are based on improving the specificity of the nanostructures to target regions of the organism, and to reduce the immunogenicity by their chemical functionalization or coating with polymers, peptides, natural polysaccharides, antibodies, and tunable surfactants.[287,300–302] If drugs do not show an affinity for a specific target or do not cross certain barriers, vehicles modified with functional groups/ligands can be used to pass through the cell membrane and allow programmed drug delivery in a particular environment.[303] Hence, it is crucial to determine the optimal ratio of receptors to ligands and to understand the interactions required to overcome the initial energy barrier for nanostructure uptake.[287,304] Noteworthy are also stimuli-responsive nanocarriers, which show the ability to liberate drugs in a controlled manner using external factors including ultrasound, heat, light, pH, and ionic strength.[305–309] They can improve the drug targeting and reduce the frequency of their dosing.

The drug delivery systems based on nanocarriers can be very complex with constituents exhibiting specific functions which can influence one another. Thus, their production should be standardized because even the slightest change in physicochemical properties may entail different targeting, leading to
toxic effects. For further clinical use, the mechanism of cytoxicity associated with a specific type of nanocarriers should be thoroughly investigated. All synthesis steps that may play a role in safety issues should be omitted. Green chemistry approaches of obtaining nanoparticles loaded with antiviral drugs are widely encouraged as they minimize utilizing the hazardous constituents and toxic solvents.\textsuperscript{[303,310]}

All in all, ideal nanocarriers should consist of biocompatible building blocks to ensure low toxicity, possess a high degree of functionality to tune host–guest interactions, exhibit a high loading capacity (e.g., high pore volume), and be synthesized in a controllable manner to precisely modulate their physicochemical properties (e.g., size and morphology). Their combination with an appropriate therapeutic drug targeting a specific disease state allows for the success of nanomedicine in the fight against viruses. Many types of nanomaterials, including liposomes, dendrimers, polymeric nanoparticles, and solid lipid nanoparticles have been applied as carriers for antiviral drugs. However, despite promising preclinical data on such therapeutic platforms, the vast majority of them fail to reach the stage of clinical trials. They are expected to be commercially successful in the near future due to the improvements in efficacy and safety of antiviral drugs.

Kraft et al.\textsuperscript{[311]} designed long-acting injectable nanosuspension of lipid nanoparticles containing three antivirals: lopinavir and ritonavir, which are hydrophobic and hydrophilic tenofovir to overcome lymph node insufficiency of oral drug combination therapy. The single injection of the formulation ensured persistent levels of antiviral drugs in macaque lymph node mononuclear cells within 1 week, while in peripheral blood mononuclear cells and plasma for 2 weeks. Furthermore, the lymphocyte-targeting properties of nanosuspension were proved by the higher intracellular drug concentrations in lymph node and peripheral blood mononuclear cells compared to those in plasma. PLGA nanoparticles containing a combination of three antiretroviral drugs (cART): efavirenz, lopinavir, and ritonavir was prepared by a high-pressure homogenization method thanks to which over 79\% entrainment efficiency of each drug was achieved. The study has shown significant uptake of the cART nanoparticles in comparison to soluble drugs and the following release of cART in the nuclear, cytoskeleton, and membrane fractions of cells without toxicity for 18 days. The higher intracellular cART delivery by nanocarriers can lead to reducing HIV-1 infectivity via inhibiting HIV replication at lower doses.\textsuperscript{[312]}

Ribavirin was encapsulated in biodegradable nanoparticles, prepared by blending of PLA homopolymer and arabinogalactan (AG)–poly(l-lysine) conjugate using the solvent diffusion method, in order to modulate the pharmacokinetics of the drug. It was found that ribavirin was accumulated in the mice livers after intravenous administration of the drug-loaded nanoparticles and, subsequently, its content slowly diminished over 7 days. The obtained nanoparticles allowed for delivery of the drug to the target organ (liver) and its sustained release.\textsuperscript{[248]} PGA polymer nanoparticles are also a promising delivery system for ribavirin to be applied in the treatment of various viral infections. The performed calculations demonstrated that ribavirin-loaded nanoparticles cause three times less accumulation of the drug in the red blood cells and 1180 times more accumulation in the liver cells compared to conventional therapy. However, the efficiency of encapsulation (≥100\%) should be improved in future studies.\textsuperscript{[313]} Stable solid monodispersed PLGA nanoparticles with diameters in the range of 50–200 nm containing ribavirin were synthesized in a microfluidic reactor with flow-focusing geometry. It was shown that the amount of the entrapped ribavirin increases upon the rising of free PLGA content in the precursor solution up to 73.6 µg mg\textsuperscript{-1} PLGA in the case of microfluidic synthesis, whereas in the bulk nanof ormulation, it was possible to introduce only 9.6 µg of drug per mg PLGA. The release of ribavirin from PLGA nanoparticles was relatively rapid.\textsuperscript{[249]}

So far, it has been found that some liposomal formulations can be beneficial for the delivery of chloroquine and ensured better suppression of parasitemia in malaria parasite \textit{P. berghei}-infected animals than the unformulated antiviral drug.\textsuperscript{[314–316]} The in vitro and in vivo stability of liposomes are changed by tailoring their size, surface charge, and lipid composition. Since they are very often absorbed rapidly by the reticuloendothelial system, they are modified with the use of different ligands and polymers extending their circulation and improving drug delivery and, consequently, also enabling clinical use. Fotoran et al.\textsuperscript{[317]} designed highly stable multilayer vesicles for encapsulation of chloroquine inducing a stronger antiparasitic effect than pure chloroquine in a lethal murine model of infection. Moles et al.\textsuperscript{[318]} used liposomes containing neutral saturated phospholipids for loading chloroquine and antibody targeting to the erythrocyte surface protein glycophorin A. This approach allowed the targeting of the nanocarrier to early intraerythrocytic stages of the malaria parasite and effective robust delivery of chloroquine to uninfected and \textit{Plasmodium }-infected red blood cells in \textit{P. falciparum}-infected mice. Baruah et al.\textsuperscript{[319]} optimized chloroquine-loaded nanostructured lipid nanocarriers employing modified double emulsion method. The nanocarriers obtained are characterized by high stability, minimal toxicity, and prevent the expulsion of the antiviral drug during storage. The in vitro and in vivo studies demonstrated enhanced antimalarial efficiency of the nanoformulations with a better suppression of parasitemia at a lower dose compared to a pure drug. Liu et al.\textsuperscript{[320]} prepared cholesterol-modified hydroxychloroquine-loaded nanocarriers to treat bleomycin-induced pulmonary fibrosis. This systemic delivery platform containing hydroxychloroquine remarkably inhibited the proliferation of fibroblasts in the rat lungs and decreased inflammation. Novel formulation of liposomal hydroxychloroquine was tested for pulmonary delivery in a preclinical study. Pharmacokinetic analysis after intratracheal administration in Sprague-Dawley rats showed that liposomal formulation enhanced 30 times the drug’s pulmonary exposure, prolonged the half-life in lungs by 2.5-fold over the intravenously and intratracheally administration of free hydroxychloroquine. At the same time, the safety profile of liposomal formulation was improved via reduction of blood and cardiac exposure of the compounds. The main limitation of this study is that the pharmacokinetics were investigated in a rat model using intratracheal instillation to simulate the intended inhalation administration, while the lung deposition efficiency with inhaled aerosols is probably lower. In addition, aerosol-generating procedures such as nebulization should be performed very carefully in patients diagnosed with COVID-19 due to the high risk of contagiousness through the respiratory
system. Sadr et al. synthesized a novel porous nanocarrier based on the magnetic Fe₃O₄ nanoparticles, utilizing 3-glycidoxypropyltrimethoxysilane as intermediate, and pectin biocompatible polymer for adsorption and release of hydroxylcholoroquine. It was shown that 32% of hydroxylchloroquine was released from the vehicle surface within 90 min reaching 75% after 5 h in a simulated human blood environment (pH 7.4) at 37 °C. The use of the Fe₃O₄@pectin composite as a nanocarrier allowed the drug release in a controlled manner. In 2021, Olejnik and Goscianska analyzed the relationship between physicochemical properties of functionalized mesoporous silica nanocarriers with different structures and hydroxylchloroquine molecules. It was found that the adsorption of the antiviral drug on the surface of nonmodified silica materials was based on the formation of hydrogen bonds, whereas, in the case of 3-aminopropyltriethoxysilane and copper ions modified samples, the complexes with drug molecules were generated. The release behavior of the hydroxylchloroquine was highly affected by the pH of the receptor fluid and the presence of functional groups on the surface of silica materials. The profile of drug liberation was the most consistent with Korsmeyer–Peppas kinetic model indicating the Fickian diffusion mechanism. The modification of ordered mesoporous silica nanocarriers with copper ions can enhance their antiviral activity.

Ritonavir was incorporated into solid lipid nanoparticles prepared with the use of solvent emulsification evaporation and double emulsion methods. The encapsulation of the antiviral drug in nanoparticles leads to its sustained release, reaching 40% in 9 days without the initial burst stage. Most likely, the extended-release is associated with drug lipophilicity and homogenous dispersion at lipid core. Moreover, the in vitro experiment indicated that ritonavir-loaded lipid nanoparticles can inhibit HIV-1 virus production like a free drug. In another study, PLGA nanoparticles were utilized as a delivery system for a combination of drugs: lopinavir and ritonavir. The drugs were either administered alone or in combination with nanoparticles to Swiss strain albino mice infected with RH virulent toxoplasma strain. Parasitological improvement in both mortality rate and parasite count was observed in the case of two forms of drugs. The higher efficiency was reached with the use of lopinavir and ritonavir encapsulated into PLGA nanoparticles which was accompanied by a minimization of the dose. Additionally, the viability and infectivity of parasites were also notably decreased. Patil and Dhawale obtained ritonavir nanoparticle formulation by nanoprecipitation technique with Eudragit RL100 polymer to improve drug dissolution and bioavailability. The complete drug release from nanosuspension within 10 min was observed and the rate of the process significantly increased in comparison to the pure drug.

3.2. Nanoparticles for Virus Inhibition

So far, metal nanoparticles have been used as therapeutic or contrast agents, but none have been shown to be beneficial for the delivery and release of antiviral drug molecules due to stable drug–metal interactions. They can directly affect the receptor binding and cell entry of viruses along with inhibition of viral proliferation.

Silver nanoparticles can inactivate different viruses, including HIV-1, monkeypox virus, murine norovirus MNV1, HSV, hepatitis B, Tacaribe virus, Rift Valley fever virus, influenza viruses like H3N2, and H1N1. Lara et al. demonstrated that Ag nanoparticles coated with polyvinylpyrrolidone exhibit anti-HIV activity at an early stage of viral replication, playing the role of a virucidal agent or viral entry inhibitor. Moreover, it was found that Ag nanoparticles produced from different fungi were capable of controlling viral infectivity by blocking the interaction of the virus with the cell. It was dependent on the Ag nanoparticle size and zeta potential. Ag nanoparticles synthesized via F. oxysporum and Curvularia species, having sizes in the range of 4–13 and 5–23 nm, respectively, exhibited better antiviral activity (80–90% inhibition) against HSV-1 and HPIV-3 and were less cytotoxic than Ag nanoparticles produced by Alternaria and Phoma species, with a size ranging from 7 to 20 nm. Green synthesized Ag nanoparticles from Lampanthus coccineus and Malephora lutea were compared in terms of potential antiviral effect. The hexane extract of L. coccineus was able to inhibit three viruses including HAV-10, HSV-1, and CoxB4, whereas M. lutea extract showed antiviral activity against only two viruses HAV-10 and CoxB4. Ag nanoparticles demonstrated antiviral and preventive effects in the infection of the H3N2 influenza virus. Kidney cells of Madin-Darby canine infected with Ag nanoparticles-treated H3N2 influenza virus displayed better viability in comparison to an influenza virus control group. Ag nanoparticles interacted with H3N2 influenza virus which resulted in destruction of morphologic viral structures in a time-dependent manner (30 min to 2 h). Spherical quasi-silver nanoparticles (size 5–15 nm) obtained with the use of an aqueous extract from Panax ginseng roots appeared to be virucidal against the influenza A virus (strain A/PR/8). Ag nanoparticles modified with zanamivir showed abilities to inhibit the neuraminidase activity of the H1N1 influenza virus. Zachar evaluated the possibility to obtain real effective minimal inhibitory concentration (MIC) of Ag nanoparticles in various respiratory system target locations during viral and bacterial infections by inhalation of standard 5 μm diameter droplets aerosol. The studies were focused on control local outbreaks of COVID-19 at-home treatment and the risk of ventilator-associated pneumonia in hospital ICU. It was found that effective MIC is attainable, both in the bronchial tree and in the alveoli. Due to the fact that respiratory infections begin in the upper airways, it is the best to use the proposed method as the first-line treatment in order to inhibit the progressive infection. 

Au nanoparticles are also widely studied in the context of nanomedicine applications due to their excellent electrical, optical, and biological features. They often act as antibacterial and antiviral agents. It was reported that Au nanoparticles conjugated with peptide triazoles (Au NP-PT) indicated a remarkably stronger antiviral activity against HIV-1 in comparison to nonmodified peptide triazoles. Changes in the physicochemical properties of nanoparticles, in particular, an increase in their diameter or the density of PT conjugated to the surface of Au nanoparticles, enhanced the inhibition of infection. The high virolytic activity and adequate irreversible HIV-1 inactivation by nanoconjugates are the result of multivalent contact between the Au NP-PT and metastable envelope.
spikes on the HIV-1 virus.\textsuperscript{[341]} Porous gold nanoparticles exhibited stronger antiviral activity against influenza A virus than nonporous sGNP and Ag nanoparticles due to their large surface area corresponding to the unique nanobundled structure. The viral inhibition occurs by blocking of viral attachment associated with membrane fusion, resulting from disulfide bond cleavage in hemagglutinin.\textsuperscript{[344]} Au nanoparticles with diameters of 2 and 14 nm functionalized with sialic-acid-terminated glycerol dendron, characterized by high stability and nontoxicity to the cell, demonstrated different antiviral activity toward the influenza virus. The studies have shown that 14 nm sialylated Au nanoparticles were very effective in the inhibition of the influenza virus while analogs with the size of 2 nm did not exhibit a significant effect.\textsuperscript{[345]} Glutathione-stabilized fluorescent Au nanoclusters were found to selectively inhibit proliferation and protein expression of porcine reproductive and respiratory syndrome virus (PRRSV) as opposed to pseudorabies virus. They directly inactivated PRRSV and blocked its adsorption without impact on viral genome replication.\textsuperscript{[346]} Zacho et al.\textsuperscript{[347]} studied Au nanoparticles coated with ligands α-terminated with sugars containing multiple sulfonate groups in terms of antiviral activity against the Dengue virus. It was shown that glucose- and lactose-based ligand exhibit a low half-maximal effective concentration (EC\textsubscript{50}) for Dengue virus inhibition, moderate toxicity, and a virucidal effect in hepatocytes.

Apart from metal nanoparticles, graphene oxide (GO) is also perceived as a potential antimicrobial material due to its unique properties such as large surface area, biocompatibility, high carrier mobility, thermal, and mechanical stability.\textsuperscript{[342,348,349]} So far, the antibacterial activity of GO has been widely explored,\textsuperscript{[350–352]} but only a few papers have presented its potential in inhibiting the entry and replication of enveloped DNA virus (herpesvirus) or RNA virus (coronavirus) in their target cells.\textsuperscript{[353,354]} GO and partially reduced sulfonated GO were effective against herpes simplex virus type 1 (HSV-1) infections. It was assumed that viral attachment blocking was the primary inhibition mechanism. Moreover, the presence of nanomaterials did not influence the cell-to-cell spread.\textsuperscript{[353]}

Carbon quantum dots (CQDs) characterized by average diameters below 10 nm, excellent water dispersion, and nontoxicity in animals are also very promising for nanomedical application.\textsuperscript{[386,355]} Their interesting optical properties enable them to be traced in vivo. It was found that CQDs can be proper scaffolds to interfere with virus entry into cells. CQDs modified using boronic acid inhibited HIV-1 entry via suppressing syncytium formation,\textsuperscript{[356]} whereas those functionalized simultaneously with boronic acid and amine groups showed antiviral activity against herpes simplex virus type 1.\textsuperscript{[357]} Ting et al.\textsuperscript{[358]} reported that uniform and stable CQDs with a size of around 1.5 nm prepared from curcumin can inhibit viral entry and the synthesis of negative-strand RNA of porcine epidemic diarrhea virus. Moreover, they were able to suppress the accumulation of reactive oxygen species by coronavirus reducing cell apoptosis. The CQDs exhibited over 50% of inhibition efficiency on virus entry at an early stage. It has been shown that the cationic CQDs can cause aggregation of the negatively charged virus through electrostatic interaction, resulting in a reduction in its infectivity.

Loczechin et al.\textsuperscript{[359]} studied the antiviral activity of different functional CQDs against HCoV-229E coronavirus. The first group of antiviral CQDs was obtained by hydrothermal carbonization of ethylenediamine/citric acid acting as carbon precursors and subsequent modification with boronic acid ligands. They exhibited virus inactivation with an EC\textsubscript{50} of 52 µg mL\textsuperscript{-1}. In turn, CQDs derived from 4-aminophenylboronic acid constituted the second group of anti-HCoV nanomaterials with an EC\textsubscript{50} reduced to 5.2 µg mL\textsuperscript{-1}. It was demonstrated that CQDs inhibit virus entry at the early stage of infection which may be the result of interaction between CQDs functional groups with HCoV-229E entry receptors (Figure 12).

Nowadays, nanotechnology offers great prophylactic and therapeutic possibilities aimed at combating various viral infections, including those caused by SARS-CoV-2. Many different functional nanoparticles provide excellent platforms for the development of completely new, safe, and effective antiviral drugs. They can also act as delivery systems for therapeutics that have already been approved. However, there are still many challenges and barriers to overcome in the near future to successfully exploit the full power of medicinal nanotechnology. It should be emphasized that the amount of nanomedicines available to patients is below all expectations. This is partly due to the translation gap between animal and human studies resulting from a lack of understanding of the differences in their physiology and pathology that affect the behavior and functionality of nanomedicines in the body. The divergence across the individual species and patients is a limiting factor in clinical translation. Amongst drug-loaded nanoparticles that have already been approved, only a few are recommended as first-line treatment options. Moreover, many of them bring about improvement in a small group of patients due to underexplored heterogeneity in the biological underpinnings of diseases which can substantially alter the therapeutic effectiveness of nanoparticles. Currently, for the controlled synthesis of nanosystems, new, advanced techniques are used allowing for the incorporation of complex architectures, bioresponsive moieties, and targeting agents to improve their delivery. Some temperature and pH-sensitive nanosystems are designed to react to the local environment and provide targeted drug delivery. The complex platforms can maximize the therapeutic efficacy, target particular phases of the cell cycle or overcome mechanisms of drug resistance.

4. Nanotechnology Potential to Inactivate Viruses under Different Conditions—Self-Disinfecting Surfaces and Personal Protective Equipment (PPE)

In view of the different routes of transmission, one of the basic and most important approaches in the fight against viruses is the prevention of their dissemination via disinfecting air, surfaces, skin, or surrounding. The design of self-disinfecting surfaces, face masks, headgear, gloves, clothes based on nanomaterials with antipathogenic properties is highly desirable. Their purpose is to prevent the contamination of the healthcare and housekeeping settings, and the entry of viruses into the human body. Moreover, new materials will be more
comfortable, safer, and resistant providing the protection against biological and chemical risks. Self-disinfecting surfaces could replace traditional chemical disinfectants (e.g., alcohols, chlorine, peroxides, quaternary amines), whose effectiveness most often requires the use of high concentrations to obtain complete viral inhibition and is limited over time. In turn, nanoengineered face masks, gloves, lab, or medical aprons can ensure new functions such as hydrophobicity and antimicrobial activity without influencing the texture or breathability of materials. The hydrophobicity of personal protective products can be an efficient barrier against airborne droplets released during sneezing or coughing (Figure 13).

For the optimization of activities aimed at reducing the spread of the current coronavirus SARS-CoV-2 with the use of available nanotechnology tools, it is necessary to know its properties, in particular, lipophilicity and surface stability under various conditions (temperature, pH, radiation).

SARS-CoV-2 belongs to group A of enveloped viruses according to Klein and Deforest classification, which are lipophilic. Therefore, it is susceptible to lipophilic disinfectants and active substances, e.g., aldehydes, halogens, quaternary ammonium compounds, alcohols, phenolics, peroxides, and proteases. These compounds are able to disrupt the surface properties of coronavirus leading to complete degradation and loss of replication functionality of the nucleic acid. Their lipophilic nature accelerates to penetrate the viral membrane and inactivation.

The previous papers have demonstrated that SARS-CoV-2 exhibits high stability at room temperature and even in cooler environments (down to 4 °C) in a wide range of pH values (3–10). However, it is sensitive to heat and undergoes inactivation after treatment at 70 °C for 5 min, like many other viruses. No significant effect on virus activity was observed after its exposure to gamma radiation. However, SARS-CoV-2 with a concentration of 5 × 10^6 TCID₅₀ mL⁻¹ was completely inactivated after 9 min of exposure to the combination of UVA and UVC radiations. It was found that UVA, used separately, was less effective in the degradation of the coronavirus. Interestingly, total coronavirus inactivation was attained rapidly after exposure only to UVC, thus confirming the reliability of this method for disinfection purposes.

It should be mentioned that SARS-CoV-2 is characterized by surface stability comparable to SARS-CoV-1. The studies performed under five environmental conditions (aerosols, plastic, stainless steel, copper, and cardboard) established viability of viruses in aerosols during 3 h and reduction in infectious titer from 10^3.5 to 10^2 TCID₅₀ L⁻¹ of air and from 10^4.3 to 10^1.3 TCID₅₀ mL⁻¹ of air for SARS-CoV-2 and SARS-CoV-1, respectively. SARS-CoV-2 shows higher stability reaching up to 72 h on plastic and stainless steel, although the virus titer was remarkably reduced (from 10^0.7 to 10^0.6 TCID₅₀ per mL of medium after 72 h on plastic and after 48 h on stainless steel). In turn, both SARS-CoV-1 and SARS-CoV-2 were inactivated on copper after 8 and 4 h, respectively. In the case of cardboard, no viable SARS-CoV-1 and SARS-CoV-2 were detected after 8 and 24 h. Moreover, it was proved that no infectious SARS-CoV-2 could be detected on treated wood and cloth after 2 days. It is also inactive on printing and tissue papers after a 3 h incubation. However, virus can remain longer on smooth surfaces (up to 4 days on glass and banknotes). Importantly, detectable level of infectious SARS-CoV-2 was still noted on the internal and external layers of a traditional surgical mask on the seventh day of studies.

Since the stabilities of both coronaviruses, SARS-CoV-1 and SARS-CoV-2 are similar, likely, nanotechnology-based methods using to inactivate the SARS-CoV-1 will also be effective in the case of SARS-CoV-2 and other enveloped viruses. Most of the approaches proposed for the inactivation of enveloped viruses are based on the optimization of the interaction between their surface and the appropriate nanosystem, thanks to which the viruses are inhibited. If a given nanosystem contains hydrophobic functional groups, they may additionally interact with lipids on the surface of the virus and support its destruction.

A novel nanotechnology-based method for the inactivation of different pathogens on surfaces and in the air using...
Engineered Water Nanostructures (EWNS) has been developed by Vaze et al. [367]. EWNS were generated via electrospray and aqueous suspension ionization of active ingredients. The proposed nanosanitizers are characterized by high surface electric charge, nanoscale size and are loaded with reactive oxygen species created during ionization of water. The EWNS platforms are able to reduce significantly viruses and bacteria concentration on surfaces and in the air. Moreover, the incorporation of hydrogen peroxide as an active ingredient into the chemical structure of EWNS increased the inactivation potency and the dose delivered to the pathogens inoculated on the surface was in the range of pico to nanogram. Such a low dose reduces the risk of chemical toxicity and makes the presented technology cost-effective which is very important for the prevention of surface and airborne nosocomial infections on a large scale.

The other methods proposed for virus inactivation are based primarily on the use of metal nanoparticles, known for their interesting intrinsic antimicrobial properties and characterized by optimal size, morphology, tunable surface charge, bioavailability, biocompatibility, and biodegradability. [364,342] Their undoubted advantage is the great possibility of decorating/anchoring/conjugating with different functional groups, bioactive molecules or linkers, which improve their effective action against viruses and other microorganisms.

Among inorganic nanoparticles, principally, Ag nanoparticles have been extensively studied due to their unique physicochemical properties and antibacterial, antifungal, anticancer, and antiviral activities. [365,368–370] They have been applied in air/water filters, antibacterial lotions, textiles, food packaging, animal husbandry, implant coatings, urinary and intravascular catheters, and as biomedical therapeutic agents in wound dressing or long-term burn care products. [342,371] Ag nanoparticles coated polyurethane condom (PUC) can efficiently inactivate HIV-1 and herpes simplex virus (HSV-1/2) infectiousness. The obtained data revealed that Ag nanoparticles-coated PUC was also able to inhibit the growth of bacteria and fungi. The novel condom developed on the basis of nanotechnology is more potent than the first-generation products. [372] Szymańska et al. [373] designed mucoadhesive gelling systems with tannic acid-modified Ag nanoparticles for effective treatment of herpes virus infections. The semisolid formulations affected viral attachment, impeded penetration, and cell-to-cell transmission, although big differences in the activity of preparations toward HSV-1 and HSV-2 were observed. They can be applied for the vaginal treatment of HSV-2 genital infection. Joe et al. [374] studied the influence of dust loading on the antiviral ability of air filters coated with Ag nanoparticles with a diameter of 11 nm. The filtration efficiency of aerosolized MS2 virus particles and pressure drop across the filter rose with dust loading while antiviral activity decreased.

Bearing in mind previous studies on the antiviral activity of Ag nanoparticles, they can also be applied successfully on various surfaces to fight the current COVID-19 pandemic. It has been reported that the antiviral activity of Ag nanoparticles against SARS-CoV-2 depends on their size and concentration. Ag nanoparticles with a size of about 10 nm at a concentration in the range of 1–10 ppm show the greatest efficiency in
inhibiting extracellular SARS-CoV-2, which can be attributed to the high stability of their interactions with viral proteins. Moreover, it was observed that they become cytotoxic to mammalian cells from the concentration of 20 ppm and above. Poly-cotton fabrics functionalized with Ag nanoparticles colloidal solution with the use of pad-dry-cure method also exhibits high anti-SARS-CoV-2 activity with an 80% inhibition rate. It was shown that Ag nanoparticles can interfere with viral replication through two different mechanisms of adhesion to the surface of the viral envelope. The conducted research opens up completely new possibilities for obtaining synthetic and natural fabrics, including cotton, polyesters, and polyamides with antimicrobial properties. Balagna et al. studied the antiviral effect of the thin silver nanocluster/silica composite coating deposited on a facial FFP3 mask toward SARS-CoV-2. It was found that composite containing the highest concentration of silver reduced the titer of coronavirus to zero. Interestingly, the inoculum persisted on the surface of the uncoated mask. The coating based on silver/silica composite can be introduced on any kind of filtering media or ceramic, glass, and metallic surfaces increasing their antiviral activity, working life besides reducing the waste products associated with disposal.

The replication and propagation abilities of SARS-CoV could be annihilated on surfaces of Ag/Al2O3 and Cu/Al2O3 catalysts exposed to air. The antiviral activity of these materials depends on oxygen access. When large droplets of the virus are present on the catalyst surface (blocking its interactions with oxygen) the viral infectivity remains unchanged. The presented approach can be applied successfully for air-disinfection in hospitals, and households.

The use of antimicrobial metallic copper surfaces can also provide protection from infectious microbes as was recently shown in many successful investigations. Normally, stainless steel is widely deployed in health care environments because of its bright, easily maintained surface, attractive aesthetic appearance, and corrosion resistance. However, there is no inherent antimicrobial advantage resulting from the application of stainless steel. In turn, copper surfaces are able to inactivate viruses, e.g., murine norovirus (MNV), bronchitis virus, poliovirus, human immunodeficiency virus type 1 (HIV-1), human coronavirus 229E, and other enveloped or nonenveloped single- or double-stranded DNA and RNA viruses. Previous reports demonstrated that MNV and human norovirus were rapidly inactivated at room temperature on copper, brass, copper-nickel surfaces, and alloys containing 60% of copper. Brasses exhibited antiviral activity against human coronavirus 229E (HuCoV-229E) and the inactivation rate was directly dependent on the percentage content of copper. Copper–nickel surfaces were also efficient in inhibiting HuCoV-229E but required at least 90% of copper content to indicate a degree of inactivation equivalent to that observed for brasses with 70% of copper. The studies of Doremalen et al. and Bryant et al. with SARS-CoV-2 demonstrated that copper alloy surfaces are much more preferable in comparison to other surfaces for inactivating coronaviruses and preventing their spread through fomite contact. Moreover, copper alloys characterized by antibacterial, antifungal, and antiviral properties have been already successfully translated into the healthcare settings allowing a 90% reduction in bioburden on touch surfaces in hospitals and about 58% reduction in infection rates observed in intensive care and pediatric units. Surprisingly, although copper alloys and impregnated fabrics are applied in healthcare and public transportation systems, they did not get much attention as a self-disinfecting surface. It is connected with the high cost of replacement of existing commonly touched objects (push plates, doorknobs, handrails, bed rails, call buttons, etc.). However, it should be mentioned that the payback time for healthcare settings installing copper alloy touch surfaces would be only several months.

This can be improved even further by considering the use of nanostructured Cu surface coatings with self-sanitizing properties or Cu nanoparticles loading onto textile fabrics which would have a significant contribution to viral infection control. UK-based Promethean Particles Ltd collaborates with textile companies and leading research laboratories to test the antiviral activity of novel Cu nanoparticles intended for use in fabrics and personal protective equipment. It was found that by embedding Cu nanoparticles into polymer fibers, such as nylon, their antimicrobial effect is maintained longer than in the case of other similar antimicrobial fabrics on the market. Bimetallic iron–copper nanoparticles (Fe/Cu NPs) form aggregates with diameters in the range of 30–70 nm, which were found to have disinfectant properties toward Escherichia coli and MS2 coliphages–surrogates of bacterial and viral pathogens, respectively. It was assumed that E. coli is strongly affected by the cytotoxicity of Cu(I), whereas MS2 is inactivated primarily due to the oxidative damages of protein capsid and RNA by Cu(II).

Kumar et al. synthesized copper@ZIF-8 core–shell nanowires (Cu@ZIF-8 NWs), which can be easily introduced on the surface of the fibers by the dip-coating method. Composites prepared via presented low-cost strategy can be successfully used in filtration media for highly hydrophobic medical face mask production. Pathogens that will reach and adhere to the surface of the mask fibers will be inactivated by the copper ions released from the Cu@ZIF-8 NWs. It was found that Cu@ZIF-8 NWs at even low concentrations exhibit antiviral properties against SARS-CoV-2 leading to 55% inhibition of virus replication after 48 h. In another study, the hybrid of shellac/copper nanoparticles was coated onto polypropylene nonwoven fabrics of commercial surgical masks. This approach enhanced the hydrophobicity and antiviral properties of the mask surface. It was demonstrated that under solar irradiation, the temperature of the mask increases, causing the generation of large amounts of free radicals. The free radicals disrupted the membrane of VLPs so that the mask exhibited a self-cleaning ability.

Respiratory protective face masks impregnated with CuO have exhibited potent anti-influenza viruses (H1N1 and H9N2) activity without change of physical barrier properties. Therefore, the development of such biocidal masks could also reduce the spread of SARS-CoV-2. Czech nanofiber technology company—Respilon is currently working on design a new face mask with natural skin-like color that will trap the viruses and at the same time destroy them. This will be obtained by pairing the textile with copper oxide particles and nanofibers. The respiratory mask will not only protect surroundings from the wearer but also the wearer from outside infection threats.

Another company Copper3D produces a face mask, NanoHack,
based on novel modular filtration system obtained from the innovative nanocomposites of PLACTIVE and MDflex. The active filtration system contains three layers of a nonwoven polypropylene impregnated with 5% CuO nanoparticles.

One of the ways to control the spread of the COVID-19 pandemic is also manufacturing self-disinfecting surfaces on the basis of the advanced oxidation processes and photocatalysis initiated by ultraviolet (UV) radiation. Methods using UV irradiation are very effective in reducing infections caused by various pathogens. Nanoparticles of titanium dioxide have been proven to act as photocatalysts and exhibit both bactericidal and virucidal properties. They are advantageous as additives for paints, air filters, and ventilation systems exposed to UV light. So far, the antiviral effect of TiO₂ has been revealed against influenza virus transmitted by aerosol resulting in respiratory tract infection, similar to SARS-CoV-2. However, the activity of TiO₂ nanoparticles for inhibition of human coronaviruses remains largely unexplored. Han et al. demonstrated that photocatalytic titanium apatite filter is able to inactivate SARS-CoV up to 99.99% after 6 h interaction without exposition to UV irradiation. In turn, under the influence of UV irradiation, the virus is completely decomposed. Another study confirmed the HCoV-NL63 virus disinfection ability of TiO₂ nanoparticles by reducing the viral genomic RNA stability and virus infectivity. The HCoV-NL63 belongs to biosafety level 2 pathogen and is very similar to SARS-CoV-2. The efficiency of TiO₂ nanoparticle nontoxic thin coatings in the inactivation of virus was maintained at multiple humid environments under brief exposure to UV, so they can be used for providing clean surfaces in healthcare and housekeeping settings. Interestingly, the Nanotech Surface Company, developed a disinfectant formulation based on TiO₂ and Ag nanoparticles, which was already applied for cleaning buildings in Milan during COVID-19 pandemic. It provides self-sterilization of surfaces from six months to two years.

Taking into account previous reports on the antiviral activity of GO (Section 3.2), it could be applied as a coating in face masks and for epidemiological exposure detection protecting health systems from the spread of the SARS-CoV-2 pandemic. The disinfection effects of GO sheets treatment on two typical enteric viruses, including EV71 (pathogenic agents of hand, foot, and mouth disease) and H9N2 (endemic gastrointestinal avian influenza A virus) were studied. It was reported that GO is an efficient label-free material that promotes complete destruction, removal, and disinfection of viruses. Chen et al. applied feline coronavirus (FCoV) and infectious bursal disease virus (IBDV) as examples of enveloped and nonenveloped viruses to investigate the antiviral activity of nanocomposites consisting of GO sheets and Ag nanoparticles (GO-Ag). The GO sheets are a supporting and stabilizing agent for silver that prevents the movements of metal nanoparticles, therefore increasing biocompatibility and reducing the toxicological effect of nanomaterial. The prepared composite inhibited FCoV infection by 25% and IBDV by 23%, while GO used without the addition of Ag nanoparticles only inhibited FCoV infection by 16% and exhibited no antiviral activity against IBDV. Further applications of antiviral composite GO-Ag for coating of face masks would minimize the risk of transmission.
of infectious agents, including SARS-CoV-2.\textsuperscript{[806]} Curcumin and β-cyclodextrin functionalized GO showed a remarkable inhibitory effect on the respiratory syncytial virus (RSV) infection. The proposed mechanisms of composite action include directly inactivating RSV, inhibition of the virus attachment onto host cells, or interfering with the virus replication.\textsuperscript{[407]}

The protective quality of face masks depends on the hydrophobicity and dryness of the external layer. If the protective layer is not resistant to pathogens, it might not provide sufficient protection and pose health risks to the users.\textsuperscript{[408]} It should be mentioned that traditional face masks have usually a gap between fibers of around 10–30 µm, which does not provide sufficient protection against viruses. Reducing this gap between individual fibers may lead to respiratory limitation and an increase in temperature and pressure, making it uncomfortable or even dangerous to wear.\textsuperscript{[407,404,409]} The constant use of face masks causes skin damage, especially among healthcare professionals.\textsuperscript{[410]} Thus, during the prevailing viral pandemic, textiles companies are increasingly looking for nanotechnological solutions and specific nanomaterials to improve existing masks. Apart from nanoparticles, also nanofibers incorporated into face masks can diminish breathing resistance, lead to the pressure drop, and additionally protect against small pathogen particles (<50 nm).\textsuperscript{[27]} The application of nanomaterials for face mask development has multiple advantages. One of the most important points is that they act both as a filter and microbiocidal agent, resulting in blocking and inactivating/ killing the pathogens. Moreover, the mask can be safely thrown away after its use because the biggest part of pathogens is destroyed in contact with the mask reducing the probability of contamination during the undressing process (Figure 13).

Nanofibers characterized by high surface area per unit mass, good interconnectivity of voids, and low weight can significantly improve the capturing efficiency of various polluting particles.\textsuperscript{[411,412]} Their functionalization with some organic groups, the above mentioned nanoparticles, and nucleating agents cause decomposition or deactivation of contaminants reducing the risk of pathogens and viruses inhalation.\textsuperscript{[413]} It was shown that the incorporation of nanofiber filter media into surgical face masks decreases airflow resistance, improving filtration efficiency in comparison to commercially available masks.\textsuperscript{[414]} The optimization of thermal comfort of face masks is also important, especially in the case of long-term use by healthcare professionals.\textsuperscript{[804]} The thermal properties of the masks largely depend on the fiber thickness. However, it should be noted that fiber thickness also affects particle removal efficiency and air permeability. The application of nanofibers on nanoporous polyethylene ensures an excellent radiative cooling effect and good particle filtration. In turn, the addition of a silver layer to these materials provides a high infrared reflectance so that they can be used for warming purposes.\textsuperscript{[415]} Wang et al.\textsuperscript{[416]} demonstrated a cost-effective method to obtain ultralightweight hierarchically structured fibrous air filter media, in which 2D nanonets run through nylon 6 and PAN nanofibers. They exhibit small pore diameters, high pore tortuosity, and can easily separate aerosol particles with a size of 300 nm. Li and Gong\textsuperscript{[417]} fabricated polysulfone nanofibers for mask filtration with the use of the electrospinning technique. It was found that these materials can efficiently filter particulate matter of 2.5 µm diameters or less while maintaining good breathability. Cellulose acetate (CA) and polyvinylidene fluoride (PVDF) nanofiber layers were applied in face masks and respirators. Their diameters were reduced by decreasing polymer concentration. CA nanofiber mats showed better filtration efficiency than PVDF nanofiber mats without increasing the differential pressure at the beginning of penetration.\textsuperscript{[418]} Electrospun nanofibers fabricated from chitosan and functionalized with quaternary amine can adsorb small viruses that can have a diameter <25 nm, e.g., a nonenveloped porcine parvovirus (PPV). The addition of graphene characterized by hydrophobicity and conductivity further improved the virus removal. The prepared system can bind even 95% of PPV.\textsuperscript{[419]} Nanocoating of antiviral polysaccharides on masks, clothing, and surfaces can also provide passive prevention of the viral spread.\textsuperscript{[420]} The nanofibrous hybrid PVDF-Ag-Al2O3 air filtration membrane obtained by electrospinning employing Ag and Al2O3 nanoparticles can kill pathogens, and at the same time detoxify different chemical compounds. It was found that an increase in Al2O3 concentration leads to a reduction in average pore diameter of the membrane. The thickness and resistance of the filter mat increase with higher loading of Al2O3 into nanofiber membranes.\textsuperscript{[421]}

Although the commonly used N95 surgical mask ensures the highest available level of protection, its filtration efficiency toward sub-300 nm particles is 85% which is caused by a wider pore size of ~300 nm. SARS-CoV-2 particles exhibit diameters of around 65–125 nm, therefore there is a need for designing more efficient masks.\textsuperscript{[422]} El-Atab et al.\textsuperscript{[422]} developed a flexible, nanoporous membrane to obtain a reusable N95 mask with an enhanced filtration efficiency. At first, a nanoporous Si-based template was prepared with the use of a combination of lithography and KOH-based isotropic etching steps, which was a hard mask during the pattern-transfer process onto a lightweight polymeric thin film. As-obtained membrane introduced into the N95 mask enhanced filtering efficiency against sub-300 nm particles, e.g., SARS-CoV-2 virus. Moreover, it was characterized by intrinsic hydrophobicity, which contributes to antifouling and self-cleaning as a result of droplets rolling and moving smoothly on the slanted mask surface.

It is worth noting that even though many different nanosystems exhibiting antiviral activity against enveloped viruses, including SARS-CoV-2, have been designed, most research is at an early stage of development. In addition to the numerous advantages opened by employing different nanomaterials, many challenges must be overcome before introducing them to the market. These challenges are generally related to the production costs, scalability, potential toxicity, side effects (e.g., skin irritation or allergy), as well as potential negative environmental impacts. Furthermore, intellectual and regulatory properties are also very important factors.

Novel reusable, self-cleaning, and efficient antiviral products based on nanostructures with super-hydrophobicity can reduce the application of conventional single-use PPE. Nanoparticle coatings on masks and protective clothing should be persistent against rubbing and washing, long-lasting, and nontoxic. A combination of self-cleaning functionalities with photothermal and photocatalytic sterilization can be their unique advantage. However, the fate of nanoparticles embedded in textiles remains unknown. Most likely, they are leached out as
nanowaste over time, potentially posing a threat to the environment. The concentration of nanoparticles that are released in washing liquids from functionalized textiles depends on their composition and production process. Decomposition products of nanostructures may also get into the wastewater and affect aquatic organisms and human life. This constitutes a big problem because due to the high surface area to volume ratio of nanoparticles, their toxicity may increase drastically, and many of the negative effects are not fully understood, which is associated with rarely conducted epidemiological studies. Some of the nanoparticles could also reach the lungs if they are not properly and heavily embedded in the face masks causing increased heart rate or decreased arterial blood oxygen pressure. Nanotechnological products that come into direct contact with the skin must be thoroughly dermatologically tested. SkinEthic reconstructed human epidermis, a fully differentiated 3D epidermal tissue constituted of normal human keratinocytes, can be successfully used for such research. Masks should be made of skin-friendly materials and exhibit good efficiency in the filtration of bio-aerosols. For the comfort of medical personnel, changing environmental conditions should also be taken into account during their design. All users of newly designed masks should be aware of the possibility of self-contamination during the removal and reuse of masks.

Currently, government agencies should establish guidelines for nanomaterial-based PPE production and provide that all manufacturers strictly adhere to them. During the course of the pandemic, the number of companies producing protective equipment has increased, along with the risk of the uncontrolled introduction of nanomaterials to the market. Therefore, it is the responsibility of national health systems to inform consumers about the health risks associated with nanowaste. In turn, users should have the necessary knowledge about the safe disposal and washing of products containing nanoparticles in order to minimize and control their release into the environment. Further studies on the application of nanotechnology for the design of more effective disinfectant and sanitizing systems, personal protective equipment, and self-disinfecting surfaces are necessary and should be stimulated on a large scale to increase control over viral infections and ensure the health and environmental safety.

5. Nanobiosensors for Diagnosis of Viral Infectious Diseases and Pathogenic Virus Detection

Given the current coronavirus pandemic and the urgency of the ongoing global healthcare debate, various methods were developed to diagnose infected people. Diagnostic methods are an indispensable tool to track the spread of the virus, control epidemics, and initiate rapid antiviral treatment. In order to detect SARS-CoV-2, a reverse transcriptase-polymerase chain reaction (RT-PCR) is frequently performed. It allows identifying the presence of viral nucleic acids in nasopharyngeal swab samples. Tests based on RT-PCR are characterized by high sensitivity, specificity, and the capability of early-stage viral infection diagnosis (Figure 15). However, at the same time, they are time-consuming (2–3 h), expensive ($5–10), and require specialized laboratory equipment supported by well-trained personnel. The diagnostic accuracy is affected by the RNA preparation step.

On the other hand, the enzyme-linked immunosorbent assays (ELISA), are used to detect the specific antibodies or antigens in the blood and to monitor immunity to infection.
or disease progression.\textsuperscript{[431]} For serological tests, low sensitivity, accuracy, and specificity are very difficult to overcome.\textsuperscript{[432]} The potential cross-reactivity of SARS-CoV-2 antibodies with antibodies induced against other coronaviruses is also a big challenge in developing precise assays for COVID-19. Additionally, it should be mentioned that changes in viral load during infection can lead to the unreliability of the diagnostic test results, whereby a higher percentage of false-negative responses is recorded (Figure 15).\textsuperscript{[216]}

Unsurprisingly, highly sensitive immunological diagnostic methods based on the use of nanobiosensors have gained global attention. Nanobiosensors characterized by enhanced biocompatibility, specificity, and stability in various media demonstrate great potential in detecting both bacteria and viruses at very low concentrations, so they can play crucial role in the case of pandemic outbreaks. They contain appropriately modified nanomaterial-based transducers to capture the selected element, transform the biological response into an electrical signal, and rapidly detect it with high precision. Among many nanostructures, in particular, metallic nanoparticles, graphene, GO, quantum dots, polymeric nanoparticles, and{CNTs} with suitable functionalized surfaces for bioconjugation and capable of signal amplification, are used in the diagnosis of viral infectious diseases and pathogenic virus detection (Figure 16). The 3D nanostructures with excellent optical, electrical, magnetic, and optomagnetic properties can significantly affect controlling and enhancing detection mechanisms of biosensors.

The most common biological components of nanobiosensors are easily recognizable enzymes, antibodies, and oligonucleotides which exhibit affinity to the specific analyte.\textsuperscript{[433,434]} For label-free nanobiosensors, direct detection of the target occurs through binding to the biorecognition element (e.g., antibody) which is immobilized on the sensing surface (Figure 17). However, here it is very important that the selected analyte provides a sufficient response in the required range of concentration, thus its molecular weight has to be large enough to induce a measurable change of signal. This means that small molecules consisting of only one epitope are not adequate for this kind of approach. They can only be analyzed indirectly by applying a competitive or inhibition detection format. In a competitive assay, the surface of nanobiosensors is covered by the biorecognition elements, whereas the analyte and its conjugated counterpart compete to be attached on the specific surface binding sites whose quantity is highly limited. Contrarily, in the inhibition detection format, the analyte-conjugate is immobilized on the nanobiosensor surface while the biorecognition constituent and analyte are added into the solution (Figure 17).\textsuperscript{[434–436]} It should be noted that proper functionalization of the nanobiosensor surface should ensure high specificity and selectivity of target analyte binding excluding nonspecific attachment or cross-reactivity toward interfering molecules which can also be present in the analyzed samples.\textsuperscript{[435]}

Biosensor platforms intended for the detection of respiratory viruses like SARS-CoV-2 are developed with three key aspects in mind: i) the target element for biological recognition (e.g., viral structural proteins, viral nucleic acid, or human immunoglobulins), ii) methods of identification (e.g., via receptors, aptamers, antibodies, probes of nucleic acid), and iii) the system of detections (e.g., surface plasmon resonance, thermal, electrochemical, optical, and mechanical systems).\textsuperscript{[437,438]}

In this section, we focus on the presentation of the most important nanobiosensors commonly applied for virus identification. Their different aspects such as advantages, limitations, and comparison with conventional sensing devices are also described in detail.

5.1. Electrochemical Nanobiosensors

Electrochemical nanobiosensors have many advantages including very high sensitivity, good stability, reproducibility, lower limits of detection, and a wider range of linear response in comparison to other sensors.\textsuperscript{[439]} The improved physicochemical features resulting from discrete nanoelectrodes or functionalization of the electrode surface with different nanostructures make these devices very attractive for pharmaceutical and medical applications. In electrochemical nanobiosensors, the detection of the signal takes place at the interface of the electrode and pathogen-containing electrolyte solution. Thus, the interaction requires a redox process followed by electron transfer in voltammetric devices (dynamic methods) while the charged species concentration, as a function of the electrochemical potential, is determined by potentiometric nanosensors (static methods).\textsuperscript{[433,440,441]} In short, the mechanism of action of electrochemical nanobiosensors relies on the change in the electrical conductance, resistance, or capacitance of the sensing surface via the nanomaterials, which occurs when the target element for biological recognition is binding to the electrode.

![Figure 16. Schematic illustration of nanobiosensor for viral detection. Transducers are integrated with different nanostructures, e.g., Au nanoparticles (Au NPs), gold nanoislands (Au NIs), graphene (GR), nanowires (NWs).](image-url)
It should be emphasized that appropriate bioreceptors must be integrated with the transducer to identify the presence of any virus in the analyzed samples. The undoubted advantage of nanobiosensors with electrochemical transduction is the possibility of their easy miniaturization into inexpensive and integrated platforms, which resemble handheld electrochemical readers useful in point-of-care diagnostics. Besides, they can be multiplexed by introducing multiple individually accessible electrodes on the same platform, enabling simultaneous detection of target elements.

Mahari et al. fabricated a cost-effective and highly specific biosensor device (eCovSens) by immobilization of nCovid-19 monoclonal antibody on the screen-printed carbon electrode for the detection of nCOVID-19 spike antigen in saliva samples. It was compared with a potentiostat-based immunosensor composed of a fluorine-doped tin oxide (FTO) electrode integrated with Au nanoparticles and nCOVID-19 monoclonal antibody. Au nanoparticles were used as a catalyst and electrochemical signal amplifiers by enhancement of electrical conductivity. Moreover, they provide a platform that enables the attachment of nCOVID-19 monoclonal antibodies as a result of electrostatic interactions or physical adsorption. Both sensors demonstrated high sensitivity for the detection of nCOVID-19 spike antigen ranging from $1 \times 10^{-15} \text{M}$ to $1 \times 10^{-6} \text{M}$ in the standard buffer. The limit of detection (LOD) in saliva samples was shown to be $90 \times 10^{-15}$ and $120 \times 10^{-15} \text{M}$ for eCovSens and FTO/Au NPs sensors, respectively. eCovSens ensures fast results within 10–30 s and requires only 20 µL of sample volume, therefore it can be applied as a diagnostic tool for detection of nCovid-19 antigen traces.

A competitive electrochemical immunosensor based on Au nanoparticle modified carbon electrodes array was also designed for detection of MERS-CoV and other coronaviruses within 20 min in spiked nasal samples. It was demonstrated that it exhibited a low detection limit of 1.0 and 0.4 pg mL$^{-1}$ for MERS-CoV and HCoV, respectively, as well as good stability. The proposed immunosensor was found to be highly selective against nonspecific proteins such as influenza A and B. Its sensitivity can be connected with using carbon array electrodes modified with Au nanoparticles causing enhanced efficiency of electron transfer.

A low-cost and highly sensitive cobalt-functionalized TiO$_2$ nanotube-based electrochemical sensor was developed for SARS-CoV-2 identification. It specifically detected the spike receptor-binding domain of SARS-CoV-2 even at a very low concentrations ($14 \times 10^{-9}$–$1400 \times 10^{-9} \text{M}$) within 30 s with a linear relationship between sensor response and protein concentration with the LOD as low as $0.7 \times 10^{-9} \text{M}$.

Pathogenic virus detection in medical samples can be performed employing field-effect transistor (FET)-based biosensing devices that enable highly sensitive and immediate measurements even at small concentrations of bioanalytes. This type of biosensor has great potential to be applied in clinical immunological diagnosis and point-of-care testing. The most frequently used nanomaterial for FET sensing platforms is graphene, characterized by high electronic conductivity, carrier mobility, and the large specific surface area, which provides an optimal environment for ultrasensitive detection. Ono et al. demonstrated the graphene FET functionalized with sialoglycan for the selective detection of the influenza virus. It exhibited very high sensitivity and specificity toward two lectins derived from *Sambucus sieboldiana* and *Maackia amurensis* used as alternatives to the human and avian virus. Another example presented microfluidic integrated reduced GO (rGO) transistor chip for detection of H5N1 influenza virus gene in the flowing environment. It was shown that immobilization of extended long capture DNA probe on the surface of rGO ensured both high stability and sensitivity. In turn, a short capture DNA probe was characterized by low stability. The developed transistor sensor based on rGO reached a LOD of $5 \times 10^{-12} \text{M}$ with a detection time of about 1 h and can be a promising candidate for the identification of nucleic acid in clinical diagnostics and environmental monitoring.

Seo et al. fabricated FET biosensor by coating graphene sheets with a specific antibody against SARS-CoV-2 spike...
protein for detecting coronavirus in clinical samples. The SARS-CoV-2 antibody was immobilized onto the sensing surface with the use of 1-pyrenebutyric acid N-hydroxysuccinimide ester as a probe linker (Figure 18). The performance and efficiency of the FET device were assessed employing antigen protein, cultured virus, and nasopharyngeal swab specimens taken from patients infected with COVID-19. It was found that the developed biosensor can detect SARS-CoV-2 spike protein at concentrations of 1 and 100 fg mL\(^{-1}\) in phosphate-buffered saline and transport medium, respectively. Its great advantage is a high-speed detection of antigens in clinical samples (>1 min) without any preparation or preprocessing while molecular diagnosis with the use of real-time RT-PCR takes at least 3 h and for loop-mediated isothermal amplification method <1.5 h. The promising results were also obtained in studies conducted in culture medium (LOD of 1.6 × 10\(^{1}\) pfu mL\(^{-1}\)) and clinical samples (LOD of 2.42 × 10\(^{2}\) copies mL\(^{-1}\)). For reference, the detection limit of PCR-based assay in sputum and for viral RNA equals 8.31 × 10\(^{1}\) and 1.50 × 10\(^{1}\) copies mL\(^{-1}\), respectively. Interestingly, the biosensor did not show cross-reactivity with the MERS-CoV antigen. Thus, the proposed highly sensitive technology can be successfully used for the diagnosis of COVID-19 and other viral diseases.

Summary of various electrochemical nanobiosensors applied for the detection of SARS-CoV-2 are collected in Table 4.

5.2. Optical and Magneto-Optical Nanobiosensors

Optical biosensors are devices in which the analyte-bioreceptor interaction causes changes in optical features such as luminescence, fluorescence, absorbance, or reflectance corresponding to the analyte concentration. They provide an alternative method of virus detection thanks to simple use, safe, and cost-effective technology. [443,457,458] So far, optical biosensors have been used to detect HIV, [459] Ebola, [460] norovirus, [461] SARS-CoV, [462] MERS-CoV, [463] and influenza virus. [464]

Surface plasmon resonance (SPR) sensing is a promising label-free method with huge potential for viral detection. The viral particles captured by the biorecognition element immobilized on the SPR sensor chip surface induce the change in the plasmon resonance wavelength or intensity measured by an optical system. [465] The general advantages of this method are high sensitivity, simple sample preparation, reusability, broad linear range, and rapid response time. Huang et al. [466] reported a one-step method for rapid and direct optical studies of SARS-CoV-2 particles with the use of a spike protein-specific nanoplasmonic resonance sensor obtained by a replica molding process. A tapered nanopillar array on a silicon wafer was employed as the original mold on which 10 nm of titanium and 70 nm of gold were deposited. It was found that the developed sensor allows detection of SARS-CoV-2 from 370 vp mL\(^{-1}\) within 15 min, while in the case of other viruses such as SARS,
MERS, and VSV, the response is negligible. The concentration of SARS-CoV-2 can be quantified linearly in the range of 0 to 10^7 vp mL^{-1}. A similar sensing capability was observed for cheap handheld optical equipment connected with the smartphone App. In addition, a dual-functional plasmonic biosensor that combines the plasmonic photothermal effect and localized SPR sensing transduction was developed for the diagnosis of COVID-19. 2D gold nanoislands self-assembled on the glass surface and modified with complementary DNA receptors provide highly sensitive detection of the selected genome sequence for point-of-care identification of viral diseases.\[458,468\] In addition, a dual-functional plasmonic biosensor that combines the plasmonic photothermal effect and localized SPR sensing transduction was developed for the diagnosis of COVID-19. However, this assay had some limitations, such as a relatively high detection limit that resulted in the misidentification of samples as a false negative. Therefore, the protocol has been improved by introducing an isothermal nucleic acid amplification step so that it can detect samples characterized by much lower viral copy numbers. Additionally, specific primer sets for the targeted region of the SARS-CoV-2 N-gene were also designed. The versatility of the presented approach was evaluated in nasal and oropharyngeal swabs; urine, plasma, and feces. The LOD was decreased to the concentration of 0.22 × 10^{-12} M\[467\].

Colorimetric biosensors are widely used to detect the target element through a color change easily detectable with a naked eye or an optical detector. Therefore, they are excellent candidates for point-of-care identification of viral diseases.\[458,468\] In order to diagnose positive COVID-19 cases from isolated RNA samples within 10 min, a colorimetric assay relied on Au nanoparticles capped with thiol-modified antisense oligonucleotides specific for N-gene of SARS-CoV-2 were applied (Figure 19). The functionalized Au nanoparticles selectively agglomerating in the presence of the target RNA sequence of the virus cause visual change in surface plasmon resonance amplified after RNaseH addition. The selectivity of the COVID-19 biosensor has been assessed against MERS-CoV viral RNA and no meaningful change in absorbance was noticed. Hence, the presented method allows for reproducible, reliable, and selective naked-eye detection of SARS-CoV-2 without the requirement of any advanced instrumental techniques.\[469\] However, this assay had some limitations, such as a relatively high detection limit that resulted in the misidentification of samples as a false negative. Therefore, the protocol has been improved by introducing an isothermal nucleic acid amplification step so that it can detect samples characterized by much lower viral copy numbers. Additionally, specific primer sets for the targeted region of the SARS-CoV-2 N-gene were also designed. The versatility of the presented approach was evaluated in nasal and oropharyngeal swabs. It was demonstrated that the accuracy, sensitivity, and specificity of the assay were >98.4%, >96.6%, and 100%, respectively, with LOD of 10 viral copies per μL. Importantly, the results of the test in clinical samples were observed by the naked eye within 40 min and no color change occurred for COVID-19 negative samples.\[459\]

Today, rapid and automated nucleic acid extraction methods are highly desirable for diagnosing COVID-19 with the use of RT-PCR. Therefore, Zhao et al.\[471\] proposed the synthesis of poly(amiño ester) with carboxyl groups (PC)-coated magnetic nanoparticles (pCMNPs) to improve the viral RNA extraction method leading to shortening the turnaround time and

### Table 4. Examples of electrochemical nano-based biosensors for detection of SARS-CoV-2.

| Nanomaterial | Target | Biological samples | Detection method | Limit of detection (LOD) | Ref. |
|--------------|--------|--------------------|-----------------|-------------------------|-----|
| Fluorine doped tin oxide electrode (FTO) with Au nanoparticles | nCovid-19 spike antigen | Spiked saliva samples; buffer samples | Cyclic voltammetry; differential Pulse voltammetry | 90 × 10^{-15} M with eCovSens; 120 × 10^{-15} M with potentiostat for spiked saliva samples; 10 × 10^{-15} M for standard buffer samples (in-house developed device) | [444] |
| Cobalt-functionalized TiO\textsubscript{2} nanotubes | S-RBD (receptor binding domain) protein of SARS-CoV-2 | CAGGS vector containing SARS-CoV-2 Wuhan-Hu-1 spike glycoprotein receptor binding domain (RBD) with a C-terminal hexahistidine tag from BEI Resources (NIAID, NIH, NR-52309) | Amperometry | 0.7 × 10^{-9} M | [446] |
| Graphene sheet | SARS-CoV-2 spike protein | Nasopharyngeal swab specimens from COVID-19 patients; cultured virus | Electrochemical field-effect transistor (FET) | 1.6 × 10^1 pfu mL^{-1} in culture medium; 2.42 × 10^2 copies mL^{-1} in clinical samples | [451] |
| Graphene sheet decorated with Au nanoparticles | SARS-CoV-2 RNA | Human throat swab specimens | Electrochemical field-effect transistor (FET) | 2.29 × 10^{-15} M for throat swab | [453] |
| Monolayer single crystal graphene on single crystal Cu(111) foils | S1 subunit protein of COVID-19 containing the RBD | S1 buffer solutions with concentrations in the range of 0.2 × 10^{-15} M–10 × 10^{-9} M | Electrochemical field-effect transistor (FET) | 0.2 × 10^{-15} M | [454] |
| Electrochemical platform made of graphene and Au NPs capped with highly specific antisense oligonucleotides | Nucleocapsid phosphoprotein (N-gene) of SARS-CoV-2 | Clinical samples (nasal swab or saliva) | Paper-based electrochemical sensor chip | 6.9 copies μL^{-1} | [455] |
| p-sulfocalix[8]arene functionalized graphene oxide; capture probes immobilized on the Au@Fe\textsubscript{3}O\textsubscript{4} nanoparticle surfaces | SARS-CoV-2 RNA | Artificial and clinical (sputum, throat swabs, urine, plasma, feces, oral swabs, serum, saliva, whole blood) RNA samples | Supersandwich-type electrochemical biosensor; Differential Pulse Voltammetry (DPV) with a smartphone equipped with a Sensit Smart electrochemical workstation | 3 × 10^{-19} M for artificial targets; 200 copies mL^{-1} for clinical specimens | [456] |
lowering operational requirements. This simplified approach
connected lysis and binding steps. The created pcMNPs-RNA
complexes were directly added into the following RT-PCR reac-
tions without elution stage and were found to be compatible
with different isothermal amplification methods (e.g., LAMP
– loop-mediated isothermal amplification, RPA – recombinase
polymerase amplification). Purification of viral RNA from mul-
tiple samples was achieved within 20 min. Moreover, a linear
correlation between 10 and 10⁵ copies of SARS-CoV-2 particles
was observed.

A comparison of different optical and magneto-optical
nanosensors used for the detection of SARS-CoV-2 is presented
in Table 5.

5.3. Piezoelectric Immunosensors

Piezoelectric immunosensors are highly sensitive analytical
devices exhibiting superior performance compared to other
biosensors. The detection mechanism exploits variations in
the resonance frequency of a piezoelectric material (PZ) corre-
lying to mass changes. If mass increases as a result of inter-
actions between biomolecules (e.g., antibody–antigen), the
frequency monitored through the alternating current voltage
decreases.⁴⁷⁶ Mass response-type piezoelectric immu-
nosensors have gained considerable attention for the virus
detection due to their ability to identify antigens in the pico-
gram range without labeling. A schematic illustration of the
piezoelectric immunosensor operation is depicted in Figure 20.
Probe antibodies are placed on the upper electrode surface of
the piezoelectric material, whose resonation is driven by upper
and lower electrodes. Then target antigen is bound by probe
antibodies. The mass change on the surface of the electrode
causes a time-dependent frequency shift that is represented
by the frequency change Δf of the material in the oscillation
circuit.⁴⁷⁶

Piezoelectric immunosensors can be successfully combined
with piezoelectric energy-harvesting devices for the IoT, giving
the opportunity to recognize viruses by controlling mechanical
vibration. Importantly, it is easy to attach or embed them into
smart clothing.⁴⁷⁶ Contemporary piezoelectric biosensors are
designed by employing new nanostructured materials and
advanced fabrication methods to avoid the toxicity of conven-
tional lead-based piezoelectric materials.⁴⁷⁸

Wang et al.⁴⁷⁹ produced a nanowell-based quartz crystal
microbalance (QCM) aptasensor for rapid and selective identifi-
cation of the H5N1 avian influenza virus. The nanoporous gold
film having a pore size of ≈20 nm was immobilized onto a gold
electrode surface utilizing a self-assembled monolayer. It was
shown that this aptasensor significantly reduced the detection
time up to 10 min without the need to apply labels. No interfer-
ence from other avian influenza virus subtypes of H1N1, H2N2,
H7N2, and H5N3 was noted.

The first piezoelectric immunosensor for SARS-CoV detec-
tion in sputum was developed by Zuo et al.⁴⁸⁰ They used horse
polyclonal antibody elicited by SARS-CoV for coating PZ crys-
tals. The antigen sample was atomized into an aerosol so it
could be specifically adsorbed by the PZ surface covered by the
antibody. The change in crystal mass led to a frequency shift
linearly dependent on antigen concentration ranging from 0.6
to 4 µg mL⁻¹. It should be noted that the sensor was charac-
terized by good reproducibility (use up to 100 times without
activity change), stability, and a short time of analysis (less than
2 min). Albano et al.⁴⁸¹ fabricated an aptamer-PQC nanobio-
sensor for the selective detection of helicase protein obtained
from SARS-CoV replication. The coupling of paramagnetic

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*Figure 19.* Schematic representation of the SARS-CoV-2 RNA selective naked-eye detection mediated by the thiol-modified antisense oligonucleotide-
capped Au nanoparticles. Reproduced with permission.⁴⁶⁹ Copyright 2020, American Chemical Society.
nanoparticle technology with nanobiosensor allowed for the identification of target proteins in assay within 1 min with LOD of 3.5 ng mL\(^{-1}\).

The interactions between the SARS-CoV-2 spike protein and the sensing surface are crucial for the detection process. Due to the fact that binding sites of the spike S1 protein are composed of hydrophobic and positively charged amino acid residues, in particular, the negatively charged surfaces should absorb/attach them through hydrophobic and electrostatic interactions. It was found that especially mixed self-assembled monolayers forming nanostructures modified with CH\(_3\) and COOH groups are the most suitable for this purpose. These engineered surfaces implemented on the QCM-based sensor can be evaluated for the diagnosis of COVID-19 reaching sensitivity up to ng level.\(^{[482]}\)

In summary, the design of modern nanobiosensors to satisfy the demands of rapid, convenient, and large-scale diagnostics of viral diseases has captured the imagination of researchers with considerable progress being made in this field. Current conventional diagnostics methods are increasingly supplanted by sensing devices constructed with the use of advanced nanotechnological solutions at a laboratory scale, and they start to penetrate successfully medical praxis. Despite the significant progress, the application of nanobiosensors in virus detection still faces many challenges that need to be considered. First, much effort must be put into decreasing detection limits. Thus, in order to overcome this limitation, different nanomaterials (e.g., Au nanoparticles, Ag nanoparticles, QDs) can be applied as labels to enhance the signal to be easily detectable. Nanobiosensor design should leverage unique optical, electrical, and magnetic features of nanoparticles, which will enable specific and selective detection of target viruses.\(^{[438]}\) Such approaches characterized by simplicity, cost-effectiveness, fast response, and real-time diagnostic procedures can replace PCR-based assays—the gold standard in the diagnosis of viral infections. It should be mentioned that nanobiosensors exhibit high stability and sensitivity at the lab scale but this can change when analyzing real samples. Many parameters will affect the obtained results, ranging from the characteristics of the target elements, the physicochemical properties of nanomaterials, to interfering molecules present in tested samples. Additionally, the nanobiosensors should be designed for the rapid identification of viruses in clinical samples, such as blood, saliva, urine, and nasopharyngeal swabs. In this context, the greatest limitation may be the costly and time-consuming procedure of extraction/preparation of the target biological elements. Hence, the fabrication of nano-based biosensors that do not require additional extraction procedures is highly desirable. Another solution can be the integration of the extraction system into the biosensor to be wearable and at the same time user-friendly.

### Table 5. Examples of optical and magneto-optical nano-based biosensors for detection of SARS-CoV-2.

| Nanomaterial | Target | Biological samples | Detection method | Limit of detection (LOD) | Ref. |
|--------------|--------|---------------------|------------------|-------------------------|------|
| 10 nm of Ti and 70 nm of Au deposited onto the nanocup array | SARS-CoV-2 spike protein | SARS-CoV-2 pseudovirus | Surface plasmon resonance (SPR) | theoretical LOD: 370 vp mL\(^{-1}\) | \(^{[466]}\) |
| 2D gold nanoslands functionalized with complementary DNA receptors | SARS-CoV-2 viral nucleic acid | RdRp-COVID, RdRp-SARS, ORF1ab-COVID, E sequence | Dual-functional plasmonic biosensor combining the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction | 0.22 \(\times\) 10\(^{-9}\) M | \(^{[467]}\) |
| Au nanoparticles capped with thiol-modified antisense oligonucleotides | RNA sequence of SARS-CoV-2 | Isolated RNA samples | Colorimetric assay | 0.18 ng µL\(^{-1}\) of RNA | \(^{[469]}\) |
| Plasmonic Au nanoparticles capped with antisense oligonucleotides | SARS-CoV-2 N-gene | Nasal swabs and saliva | Nanoamplified colorimetric assay | 10 copies µL\(^{-3}\) | \(^{[470]}\) |
| Poly(amino ester) with carboxylic groups (PC)-coated magnetic nanoparticles (pcMNPs) | Viral RNA extraction of SARS-CoV-2 | SARS-CoV-2 pseudovirus diluted in fetal calf serum | Fluorescence; Conventional RT-PCR protocol | 10 copies of pseudovirus | \(^{[471]}\) |
| Functionalized Au nanoparticles conjugated with the SARS-CoV-2 antibody | SARS-CoV-2 spike protein | buffer solutions of spike proteins with different concentrations (4-12 fmol) | Plasmonic biosensor device based on toroidal dipole-resonant metamolecules | 4.2 fmol | \(^{[472]}\) |
| Au nanospikes covered glass substrate | Antibodies specific to the SARS-CoV-2 spike protein | Human plasma diluted in 1 mL of buffer solution | Localized surface plasmon resonance (LSPR) | 0.08 ng mL\(^{-1}\) (≈0.5 \(\times\) 10\(^{-15}\) M) | \(^{[473]}\) |
| Anti-SARS-CoV-2 functionalized Au nanoparticles | Surface proteins of SARS-CoV-2 (spike, envelope, and membrane) | Nasal and throat swabs | Colorimetric assay | \(C_L = 36.5\) (detection limit of the biosensor was reported based on the real-time PCR cycle threshold – \(C_L\)) | \(^{[474]}\) |
| Functionalized streptavidin-coated iron oxide nanoparticles | SARS-CoV-2 RdRp (RNA-dependent RNA polymerase) coding sequence | Target DNA in buffer solution containing 10% of fetal bovine serum | Optomagnetic sensing | 0.4 \(\times\) 10\(^{-9}\) M | \(^{[475]}\) |
The designed platforms could be translated into microneedle-based biosensors that permit for continuous monitoring of SARS-CoV-2 antigens, antibodies, or nucleic acid in the dermal interstitial fluid of population infected by coronavirus symptomatically and asymptomatically. Wearing such devices would provide key information about the spread of viral infection in a community, the development or loss of immunity, and the immune response to vaccination. It should be noted that antibody levels fluctuate over time, which may be due to natural infection or vaccination. Continuous measurements would help to track these changes, indicating a need for a booster vaccination. Additionally, it would be possible to predict the likelihood of subsequent waves of the pandemic.

It is expected that the collaboration between researchers, engineers, and industrial partners will allow the technological breakthrough necessary to bring nanobiosensors to the medical community. These sensors will overcome false negative/positive results, validation processes, cost-effectiveness, detection speed, and selectivity. Future efforts should focus on developing innovative, fast, affordable, and miniaturized biosensing devices for diagnostic applications to control the spread of viral infectious diseases.

6. Conclusion

In this review, we aimed to critically review the current situation of the nanoscience research against viruses and estimate the impact it can bring to society. This pronouncedly interdisciplinary field has been burgeoning over the last 15 years, and especially expanded in the last 2 years (Figure 2). Over the years, nanoscience has proven to be extremely powerful in four main areas: i) nano-based vaccines, ii) nanocarrier-based drug delivery system, iii) personal protection equipment such as masks, gloves, and self-disinfecting surfaces, and iv) nanobiosensors.

Nanotechnology-based platforms for vaccines and drugs are the key milestones in medicine and biotechnology opened by nanoscience, enabling the efficient loading and safe delivery of the targeted species (e.g., mRNA, antivirals). Another important research directions advanced by nanoscience are the fields of nanobiosensors and self-disinfecting materials (e.g., surfaces, face masks, gloves, clothes) based on nanomaterials with antipathogenic properties, which both show excellent potential for improving current solutions. Although those fields have promising performances at the laboratory scale, when it comes to bringing them to the market, one should consider how to make them better, cheaper, and more reliable than state-of-the-art solutions. Nanoscience research can make a great impact in medicine but has to offer elegantly simple solutions based on an inexpensive, reproducible, and high-performance synthesis of the nanoformulations that can be scaled-up by pharmaceutical companies and even modified for other challenges (e.g., other viruses). Therefore, a collaboration of interdisciplinary scientific groups with industry is necessary to reach both a fundamental understanding of nanosystems in the context of viruses and at the same time solve the challenges that might arise during translation from the laboratory to the clinics.

All in all, with the fright in the world, arising due to the viruses, and SARS-CoV-2 in particular, researchers have to
take it as a responsibility to benefit society and act accordingly. Although vaccines against SARS-CoV-2 will strongly decrease the number of new cases, viruses will not disappear. To be prepared for new pandemics, scientists should focus on the improvement of drug delivery platforms that have been proven to be a strong tool to fight against viruses. It is beyond the question that nanoscience can and will make contributions to combat viruses, but the key challenge will be that those contributions are not only scientific but importantly social.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Author Contributions**

J.G. contributed to conceptualization, writing the original draft, writing review and editing, and assisted in visualization. R.F. also assisted in visualization. S.W. contributed to conceptualization and writing the original draft.

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Joanna Goscianska is an associate professor at the Faculty of Chemistry of Adam Mickiewicz University in Poznań (AMU). She obtained a Master’s degree in 2005, a doctoral degree in 2009, and habilitation in 2019, in the field of chemistry. During her Ph.D. studies, she participated in three research internships at Laboratoire Catalyse & Spectrochimie in Caen (France). In 2018, she became a laureate of the habilitation fellowship “L’ORÉAL-UNESCO FOR WOMEN IN SCIENCE.” Her principal interest is focused on synthesis, modification, characterization of porous materials (e.g., metal oxides, ordered mesoporous silica and carbons, metal–organic frameworks) and their application in adsorption processes, catalysis, and drug delivery systems.

Stefan Wuttke created the research group “WuttkeGroup for Science,” initially hosted at the Institute of Physical Chemistry at the University of Munich (LMU, Germany). Currently, he is an Ikerbasque Professor at the Basque Center for Materials, Applications and Nanostructures (BCMaterials, Spain). His research is focused on developing methodologies to write and read chemical information onto and from the backbone of hybrid framework materials (including framework nanoparticles). In addition, his research interests also include the acquisition of a fundamental understanding of the chemical and physical processes involved in their synthesis and functionalization.