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Role of Nanotechnology in Diagnosing, Safeguarding, and Treating COVID-19

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ABSTRACT: Coronavirus disease (COVID-19) is arguably the worst health crisis the world has faced in the 21st century, and the World Health Organization declared this a "public health emergency of international concern" during the beginning of the pandemic and continued for a significant period of time. Considering the public health risk and the delay in introducing a suitable medical intervention to eradicate this virus, many reserachers embarked on different technologies to develop a cure. Nanotechnology has emerged as a promising weapon in the fight against COVID-19 and other similar viral diseases. The unique qualities of nanomaterials make them excellent for a variety of applications, including the development of low-cost, real-time diagnostic systems, reusable personal protective equipment, and innovative carriers for biological cargo such as mRNA in vaccines and CRISPR/Cas9 in gene editing. In this review the current available pharmacological and non-pharmacological options that are being used around the world against COVID-19 are compared with their nanotechnological counterparts. Here, we also elaborate the advantages of currently available nanotechnology-based diagnostics, protective equipment, vaccines and therapeutics and discuss future directions and steps that should be taken to translate these technologies into a clinical setting to combat the COVID-19 pandemic.

Keywords: Covid19; SARS-CoV-2; Nanotechnology; RNA Vaccine; Lipid nanoparticles.

دور تقنية النانو في تشخيص وحماية وعلاج COVID-19

كينيث هولوغالا ، بانشالي راناسينغ ، غاريث ويليامز ، ك.م. نالين دي سيلفا ، وروهيني م. دي سيلفا

الملخص: يمكن القول إن مرض فيروس كورونا (COVID-19) هو أسوأ أزمة صحية واجهها العالم في القرن الحادي والعشرين ، وقد أعلنت منظمة الصحة العالمية أن هذه "حالة طوارئ صحية عامة تثير قلقًا دوليًا" خلال بداية الجائحة واستمرت لفترة طويلة من الوقت. بالنظر إلى مخاطر الصحة العامة والتأخير في إدخال تدخل طبي مناسب للقضاء على هذا الفيروس ، شرع العديد من الباحثين في تقنيات مختلفة لتطوير علاج ، ظهرت تقنية النانو كسلاح واعد في مكافحة COVID-19 وغيرها من الأمراض الفيروسية المماثلة. الصفات الفريدة للمواد النانوية تجعلها ممتازة لمجموعة متنوعة من التطبيقات ، بما في ذلك تطوير أدوات تشخيص منخفضة التكلفة في الوقت الحقيقي ، ومعدات حماية شخصية قابلة لإعادة الاستخدام ، وناقلات مبتكرة للبضائع البيولوجية مثل mRNA في اللقاحات و CRISPR / Cas9 في الجينات. في هذا البحث ، تتم مقارنة الخيارات الدوائية وغير الدوائية المتاحة حاليًا والتي يتم استخدامها في جميع أنحاء العالم ضد COVID-19 مع نظيراتها في مجال التكنولوجيا النانوية. هنا، نوضح أيضًا مزايا التشخيصات القائمة على تقنية النانو ومعدات الحماية واللقاحات والعلاجات المتاحة حاليًا، ونناقش الاتجاهات والخطوات المستقبلية التي يجب اتخاذها لترجمة هذه التقنيات إلى بيئة سريرية لمكافحة وباء COVID-19.

الكلمات المفتاحية: كوفيد 19 ، سارس كوف-2 ، تقنية النانو ، لقاح الحمض النووي الريبي، الجسيمات الثانوية الدهنية.

1. Introduction

The COVID-19 pandemic caused by the SARS-CoV-2 virus is arguably the worst health crisis faced by humanity during the 21st century, exerting a pervasive effect on all aspects of human life in almost every country worldwide. As of January 2023, more than 650 million cases have been recorded in a total of 222 countries, with over 6 million deaths [1]. The effects of SARS-CoV-2 stunned experts and the general public alike, primarily since it broke the patterns seen with all other recent coronaviruses such as SARS-CoV-1 in 2002 and MERS-CoV in 2012 [2,3]. These viruses are the closest comparisons to the covid 19, due to their chronological proximity and inclusion in the same family, but their impacts vary drastically. The SARS-CoV-1 virus was responsible for 774 deaths from 8096 reported cases between 2002 and 2004, having a case fatality rate (CFR) of 10%. The MERS virus showed a much higher CFR, with 850 deaths from 2400 reported cases for a CFR >35% [2]. The global CFR of SARS-CoV-2 pales in comparison, ranging between 2-4% currently, with some countries having CFR values as low as 1% (comparable to the typical influenza virus) and other developing countries having values as high as 25% (particularly due to the collapse of healthcare systems) [4]. However, this information is misleading with regards to the seriousness of SARS-CoV-2; albeit being less fatal than previous coronaviruses, it is much more contagious and widespread, leading to a truly global impact with social distancing steps, mask mandates and lockdowns being necessitated in many countries to control the virus [5].

Dry cough, loss of taste and smell, fever, body aches, weariness, and potentially fatal acute respiratory distress syndrome (ARDS) are all symptoms of SARS-CoV-2 [6]. The virus mainly attacks the lungs, but it also has the ability to affect other organs such as the cardiovascular system, central nervous system, kidney and liver, [7] particularly in immunocompromised patients and individuals with comorbidities (e.g. diabetes, cancer, cardiovascular diseases, neurological diseases) [4]. Vaccines against the virus hit the market in December 2020,[8] but it has constantly mutated to result in five major variants from different parts of the world including the UK (Alpha), South Africa (Beta, Omicron), Brazil (Gamma) and India (Delta) [9,10]. The Delta variant caused more severe disease than other variants in people who were not vaccinated. The procurement and availability of vaccines in developing countries has also been a major hurdle in combating the coronavirus and achieving herd immunity, with 69.5% of the world's high income countries vaccinated with at least one dose as of January 2022, but with low income countries severely lagging with a meagre 10.9% vaccinated with at least one dose [11]. Therefore, unless there is coordinated global action to improve vaccine equity, the probability of the emergence of a vaccine-resistant variant is ever present.

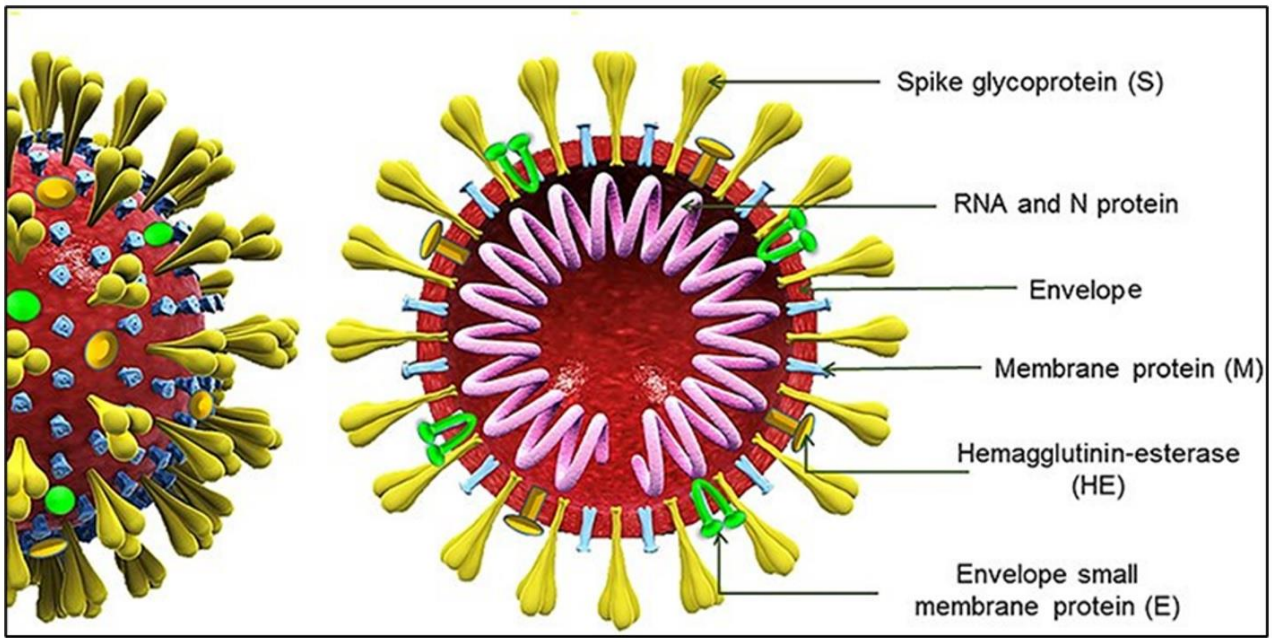

Figure 1. Schematic diagram of the SARS-CoV-2 virus showing the assembly of the genome (RNA and nucleocapsid protein) and the envelope (Spike glycoprotein (S), envelope protein (E) and membrane protein (M)).

Due to the introduction of new strains and mutations, no effective treatment has been developed yet, with a range of antiviral and anti-inflammatory drugs being repurposed for relief of symptoms with varying degrees of success [9]. To overcome the limitations of traditional systems, a truly multidisciplinary approach is required, and SARS-CoV-2 has brought together scientists from every discipline. Nanotechnology and nanomedicine offer a promising and proven alternative to conventional therapies, as exemplified by the effectiveness of nanoparticle based therapies for the treatment of other diseases, particularly cancer [12]. The chances of nanotechnology being a successful approach against COVID-19 are also high due to several factors; SARS-CoV-2 is similar in size (60-140 nm) to most of the current Food and Drug Administration (FDA) approved nanodrugs, paving the way for "direct combat" therapy, [13] nanoparticles have been shown to enhance the stability of mRNA vaccines [14], and the physicochemical properties of nanomaterials can be tuned to combat viruses [15]. These are a few potential uses of nanotechnology in combatting COVID-19, but the opportuntities for the utilization of nanotechnology in the fight against the virus are in fact far greater, with applications in respiratory masks, gloves, personal protective equipment (PPE), air filtration systems, disinfectants, and detection kits for diagnosis [16]. This review focuses on the use of nanotechnology under the categories of treatment (e.g., nanoscale drug delivery systems), prevention (e.g., PPE, disinfectants and vaccines) and diagnostics (e.g., rapid detection assay kits). We critically discuss the advantages, shortcomings and future prospects for nanotechnology in the setting of COVID-19. This review comprehensively considers the current knowledge of the subject and will help researchers and scientists to formulate innovative strategies to mitigate this ongoing pandemic.

2. The structure of SARS-CoV-2 and its effect on the human body

2.1 Structure of SARS-CoV-2

The typical architecture of a virus comprises a virus-coded protein coat (envelope) that protects the viral genome, which could be made up of either RNA or DNA [17]. Accordingly, as shown in Figure 1, [18] the SARS-CoV-2 virus contains an envelope consisting of spike protein, envelope protein, and membrane proteins [19]. This envelope also shares the common feature of other coronaviruses, having a crown-like appearance [19] due to the presence of spike proteins made out of glycoproteins [20]. The genome contains a positive-stranded RNA molecule assembled with nucleocapsid protein [21]. The receptor-binding domain of the envelope shows an affinity towards the angiotensin-converting enzyme 2 (ACE2) receptors in the lower respiratory tract [20].

2.2 Effect of SARS-CoV-2 on the human body

The SARS-CoV-2 virus can spread COVID-19 to the general public in a variety of ways. Anyone can become infected if the minute liquid particles discharged from an infected person's mouth or nose contaminate their nose, mouth, or eyes [22]. According to case study reports, key symptoms can be classified as (1) otolaryngological (e.g. loss of sense of smell and taste, sore throat, cough, nasal congestion, earache, runny nose, tinnitus, hoarseness, etc); [23] (2) neurological (e.g. headache, dizziness, impaired consciousness, unstable walking, cerebral hemorrhage, cerebral infarction, etc); [24] (3) psychotic (e.g. delusions, orientation/attention disturbances, auditory and visual hallucinations, etc); [25] and, (4) dermatological (e.g. erythematous rash, urticaria and chicken pox-like lesions) [26].

When the viral genome is replicating, the monocytes in the host's alveolar space secrete proinflammatory cytokines, chemokines and induce pneumocytes apoptosis. Additionally, the chemokines and cytokines released by recruited macrophages promote capillary permeability and neutrophil recruitment. Due to neutrophil degranulation, the alveolar-capillary barrier is broken, causing irreparable impairment to pneumocytes and endothelial cells. The generation of neutrophil extracellular traps (NETs), in which the neutrophils release their intracellular content (mainly DNA and proteins) into the extracellular compartment as a trap to apprehend pathogens, is also an important factor. Irrespective of the mechanism, ultimately blood proteins transmigrate, causing interstitial and alveolar edema [27]. Table 1 details a summary of the pathological conditions of several organs affected by COVID-19.

Table 1. Pathological conditions of the lungs, heart, placenta, kidney and gut caused by COVID-19.

Organ	Pathological conditions	Reference(s)
Lungs	• Become deteriorated to the stage clinically known as acute respiratory distress syndrome (ARDS) • The histopathology leading to ARDS has been identified as diffuse alveolar damage (DAD), in which the capillary endothelial cells and alveoli epithelial cells are permanently impaired with protein-rich fluid leakage into the interstitial and alveolar space • Subsequent formation of hyaline membrane and intracapillary thrombosis causes surfactant stabilization, alveolar collapse, and	[27]

	ultimately hypoxemia	
Heart	• Myocarditis • Inflammation in blood vessels • Cardiac arrhythmias • Acute coronary syndrome (ACS) – COVID-19 associated thrombophilia – release of proinflammatory cytokines (IL-6) – exacerbation of earlier cases of severe coronary artery disease – stress cardiomyopathy – reduced coronary circulation • impaired oxygen supply causes the destabilization of coronary plaque microthrombogenesis	[21]
Placenta	• Molecular pathological tests have detected SARS-CoV-2 antigens in the villous syncytiotrophoblasts on the villous chorion, chronic histiocytic intervillositis, and syncytiotrophoblast necrosis • The virus has not been detected within Hoffbauer cells in the placenta	[28] [29]
Kidney	• Direct cytotoxic injury from the virus • Imbalance in the renin-angiotensin-aldosterone system • Cytokine-induced hyperinflammatory state • Microvascular injury • Hypercoagulable state • Hypovolemia • Kidney injury due to potential nephrotoxic agents and nosocomial sepsis	[21]
Gut	• Shedding of the mucosal epithelium • Erosion of the intestinal mucosa • Focal inflammatory necrosis with haemorrhage • Massive neutrophil infiltration and macrophage proliferation with minor lymphocyte infiltration • Microbiological observations have identified fungal spores and Gram-positive cocci • Detection of RNA of SARS-CoV-2 virus in intestinal macrophages supports the conclusion of possible gastrointestinal infection	[30]

3. Current management of COVID-19

Even after SARS-CoV-2 was declared a pandemic by the World Health Organisation (WHO) in March 2020, [31] there has been a dearth of drugs approved for the treatment of the virus, and not a single drug has been discovered solely for the purpose of combating COVID-19. The US Food and Drug Administration has already approved Remdesivir (an antiviral drug originally developed to treat hepatitis C and Ebola) [32]. Several other immunomodulatory medicines, notably Tocilizumab for the treatment of COVID-19 patients with severe symptoms necessitating hospitalization, have also received emergency use authorisation [33].

Non-pharmacological therapies have been the cornerstone in the care of COVID-19 due to a lack of appropriate medicines. These include preventative measures such as social distancing, hand washing, using face masks, and reducing public gatherings, among others [34]. Non-pharmacological treatments also include supportive therapies like oxygen therapy for those patients presenting with more severe symptoms and requiring hospitalization [27,35].

3.1 Pharmacological management

There has been a definite increase in the understanding of how different drugs affect the virus as well as better understanding of the risk/reward ratio in the use of certain drugs. For example, there were studies in the initial days of the pandemic that advocated the use of medications like hydroxychloroquine, lopinavir/ritonavir, and ivermectin to treat COVID-19 patients [36-38]. However, following the gathering of new information, the WHO issued strong recommendations against using these medications in patients with COVID-19 of any severity [39]. The WHO now strongly recommends the use of IL-6 receptor blockers (tocilizumab or sarilumab) and corticosteroids in patients with severe or critical COVID-19 infection [39].

This is apart from the general antiviral treatments like vitamin C and D, zinc, and selenium which are used as immune boosters in COVID-19 patients [40].

Remdesivir, an antiviral medication that works by blocking viral RdRP (RNA-dependent RNA polymerase), was shown to be selectively cytotoxic against SARS-CoV-2. It is one of the first drugs which have been used to treat COVID-19 patients belonging to distinct categories such as pregnant women and immunocompromised individuals [41,42]. There is also an abundance of evidence in the form of case studies and clinical trials that have established the efficacy of this anti-viral drug [43]. It is currently being used as a COVID-19 treatment option for both adults and paediatric patients above the age of 12 years after hospitalization [44]. Another FDA-approved therapy is casirivimab/imdevimab (REGEN-COV), a recombinant monoclonal medicine or antibody cocktail that specifically targets the RBD region of the SARS-CoV-2 spike protein [45]. Even though the clinical data pertaining to casirivimab is limited, it has so far shown lowering of viral load in patients at the initial stage of infection or a baseline viral load [46]. Antiviral and antiparasitic agents are two classes of repurposed drugs that have been employed to tackle the COVID-19 pandemic. Most of these anti-viral drugs have been used in clinical practice during SARS-CoV-1 and MERS outbreaks and have been identified as promising therapies against COVID-19 [44]. Lopinavir and ritonavir are used in combination for anti-HIV retroviral treatment, as well as being clinically tested as a SARS-CoV therapeutic intervention [47]. The active period of lopinavir or the exposure period in patient plasma is improved by ritonavir, which functions as an efficacious inhibitor of host proteases (particularly the p-glycoprotein efflux mediator and cytochrome P450 3A4 enzyme) [44,47]. A study done during SARS-CoV-1 showed a curtailing of intubation levels and mortality rate upon use of this drug combination [48]. So far, lopinavir/ritonavir has shown mixed results as a therapy for COVID-19. Some studies indicate quick improvement and significant viral load clearance [38], whereas other clinical trials have concluded that it does not offer clinical improvement or diminished mortality rates compared to standard care [49]. As a result, further experimental and clinical trials should be conducted in order to gather indisputable proof.

Corticosteroids such as dexamethasone and methylprednisolone are currently used as supplementary drugs to alleviate the severe inflammatory responses which are often associated with Accute Respiratory Disease Syndrome (ARDS) and COVID pneumonia [44,47]. Multiple clinical studies have shown the benefits of corticosteroid therapy, such as the removal of ventilation and higher chances of extubation in patients. While more clinical trials are needed to determine the efficacy of corticosteroids in COVID-19 patients, their ability to combat ARDS symptoms has made them an effective therapy management strategy thus far [50,51,52].

Tocilizumab (TCZ) is a monoclonal antibody that inhibits the interleukin-6 receptor (IL-6R) and is commonly used as a therapeutic immunosuppressant. As a result of its success in treating COVID-19, it was approved by the FDA as an emergency use therapy and by the WHO for the treatment of patients with severe COVID-19, as stated previously. It is often used to treat COVID-19 patients' hyperinflammation and other immunological reactions [44]. A range of clinical trials have thrown light on the efficacy of tocilizumab in patients, with strong reduction in inflammatory biomarkers, relief of cytokine storm in the respiratory system, scaling down of the requirement for intubation and cessation of clinical collapse [33,53-56].

Among the other clinically studied and ongoing trials of supplementary treatments are studies on therapeutics that lessen the immune response or immunomodulators such as IFN-α2b, IFN-β1b, and IFN-β1a. IFN-α2b, in particular, has also been shown to cause a reduction in inflammatory cytokines such as IL-6 and CRP in the period of time over which virus can be detected in the upper airways of patients [57]. Similarly, IFN-β1b has aided in the process of reducing mortality and improving discharge rate as well as giving positive progress in clinical wellbeing [58]. Tissue plasminogen activator (tPA) is another type of supportive therapy which is usually administered to COVID-19 patients with high thrombus formation risk. It has been proven that this therapeutic is capable of instantaneous dissolving of the thrombi, alongside ameliorating the respiratory condition of the patients [59].

As part of a comprehensive pharmacovigilance strategy, the efficacy and safety of existing COVID-19 therapies should be closely monitored. Efficacy and safety data for several repurposed and supporting medications have been mixed. Multiple methodological flaws make it difficult to evaluate results from studies, the most prominent of which is the lack of a reliable COVID-19 control therapy. Antiviral therapy and a variety of supportive treatments, such as immunomodulation and antibody supplementation, will likely continue to play pivotal roles in COVID-19 treatment. While new therapies are being developed, we can only hope that careful usage of the currently available medications will continue to help patients [44].

3.2 Non-pharmacological management

The basic motive behind non-pharmaceutical interventions is to curb the transmission of SARS-CoV-2 infection. Social distancing and lockdowns have been practiced with the main objective of reducing the Rt value (time varying reproduction number) below 1. Even though these drastic management decisions have created the desired effect, it has been achieved only with a pronounced socio-economic detriment [60].

Supportive treatments are another category of non-pharmacological management used for COVID-19 patients. The most common are oxygen therapy, the prone position, and nitric oxide inhalation [61].

Under oxygen therapy, respiratory support is provided to hypoxemic patients where blood oxygen level is improved and maintained at least above 90% [62]. Non-invasive ventilation is an assisted respiratory support technique which is used to avoid the difficulties faced during intubation [64]. It is usually approved for patients at early stages of COVID-19 infection and also for individuals with milder immunosuppressive and cardiovascular complications but it only delays the invasive ventilation [65,61].

Invasive ventilation involves positive pressure gradient ventilation directed via an endotracheal or tracheostomy tube. The most common techniques are synchronized intermittent mandatory ventilation (simv), pressure support (ps), positive end expiratory pressure (PEEP) and continuous positive airway pressure (CPAP) [63]. Extra corporeal membrane oxygenation (ECMO) is an advanced invasive ventilation technique typically recommended for individuals with limited organ failure and good premorbid functional status. Since this is a final stage intervention, it is initiated only after the failure of other ventilation support, and after weighing the risk- benefit trade-off [65]. Another advanced invasive ventilation strategy used to avoid intubation in patients with respiratory arrest and severe hypoxemia is the use of a high-flow nasal cannula. This allows sufficient tissue oxygenation of patients with acute respiratory failure [66].

The prone position is a technique which improves ventilation perfusion by repositioning the heart in the chest to better recruit pulmonary alveoli. This has been proposed as an adjunct to other supportive therapies and several studies have shown that use of the prone position in COVID-19 patients increases oxygenation [34,67,68].

From the results of current research, there is no specific non-pharmacological treatment that can be said to have great effectiveness against COVID-19. Studies have been conducted with small cohorts and the results are largely skewed, while the evidence is indirect. However, several approaches do show potential and there is a need for more rigorous clinical trials to be conducted to establish more reliable evidence with regard to non-pharmacological treatments.

4. Nanotechnology in COVID-19

Nanotechnology has infiltrated practically every subspecialty of research, including chemistry, biology, medicine, agriculture, the food industry and cosmetics, since its inception. Nanotechnology has shown promising results in treating viral infections such as HIV, herpes simplex and respiratory viruses [69]. During the COVID-19 pandemic, nanotechnologists have played critical roles in multiple aspects, from diagnosis through treatment, prevention, and vaccine development [70]. Nanotechnological strategies can be used either internally or externally to the human body to combat the disease. Some novel products are already in use by healthcare professionals and the general public, while others are still in clinical trials. The notable characteristic of nanotechnology is that it can be engineered in such a way to avoid the drawbacks and enhance the preferable qualities of already available products [71]. The integrative approach of nanotechnology in the diagnosis of, and protection and treatment against COVID-19, along with its uses in vaccine development, will be discussed in this section.

4.1 Nanotechnology in diagnosis

COVID-19 diagnosis is critical in the fight against the virus and is required to prevent the virus from spreading further and increase the chances of saving lives through supportive care. Chest computed tomography (CT) imaging, chest X-ray, nucleic acid-based methods (real-time polymerase chain reaction, RT-PCR), and immunoassays or serology tests (enzyme-linked immunosorbent assays, ELISA) are used in clinical practice to diagnose COVID-19 [72,21]. Despite its considerable drawbacks, RT-PCR is the predominantly used technique for diagnosis. The main drawbacks include the inability to detect asymptomatic patients because it requires a comparatively high virus load (high lower detection limit) to declare as positive for COVID-19, a lack of sufficient technical support (including laboratory facilities in rural or undeveloped areas of the world), and the unavailability of RT-PCR kits to fulfil the demand for tests. Nanotechnology has helped to alleviate some of the existing challenges [73].

One-step reverse transcription loop-mediated isothermal amplification coupled with a nanoparticle-based biosensor assay (RT-LAMP-NBS) and reverse transcription loop-mediated isothermal amplification combined with chemiluminescence (RT-LAMP-CL) are two techniques that eliminate the need for costly PCR machines and the prolonged thermocycling times used in current PCR techniques [74]. RT-LAMP-NBS has successfully combined nanotechnology and the RT-PCR technique. The temperature of the LAMP primer sets, F1ab (opening reading frame 1a/b), and nucleoprotein genes of SARS-CoV-2 has been kept constant at 63^0C for 40 minutes to isothermally amplify the nucleic acid molecules simultaneously. The nanoparticle-based biosensor has easily interpreted the results. According to the results, the sensitivity of the test has been 12 copies per reaction and there has been no cross-reactivity from other non-SARS-CoV-2 templates. From sample collection to result interpretation, this biosensor took approximately an hour, demonstrating the qualities which make it a potential novel biosensor [75]. Similarly, using nanotechnology, RT-LAMP can be paired with chemiluminescence to detect the presence of the virus by the emission of a chemiluminescence

signal. Here, the chemiluminescence signal is generated by a reaction between streptavidin modified alkaline phosphate (ALP) enzyme and biotin-labeled amplified products [74,73].

In clinical COVID-19 diagnosis, a dual-functional plasmonic biosensor with the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction could be a viable alternative to the RT-PCR approach. In this innovation, the detection of SARS-CoV-2 is achieved by employing the nucleic acid hybridization theory. Detection happens upon binding of chosen sequences from SARS-CoV-2 to two-dimensional gold nanoislands (AuNIs) which are functionalized with complementary DNA receptors. Thermoplasmonic heat is generated when the AuNIs chip is illuminated at the plasmonic resonance frequency, resulting in an effective diagnosis. Even when the RNA concentration is as low as 0.22 pM, this biosensor has a strong affinity for pre-selected viral genome sequences, resulting in accurate detection in a multigene mixture [76].

A field-effect transistor (FET)-based biosensing device is another promising alternative to the RT-PCR technique. This device detects the presence of SARS-CoV-2 in swab specimens collected from the nose and throat via an antibody reaction mechanism. A distinct antibody against the SARS-CoV-2 spike protein was placed on a spin-coated graphene sheet to create the device. The results have shown that the lower detection limits of the device are 1 fg/mL when the sample is in phosphate-buffered saline, 100 fg/mL when in a clinical transport medium, 1.6 × 101 pfu/mL in culture media, and 2.42×10^2 copies/mL in clinical samples. Since it does not require sample pre-treatment or labeling, this device is far superior to the traditional RT-PCR technique [77].

Another nanotechnological intervention has resulted in a colorimetric biosensor using gold nanoparticles (AuNPs) to detect SARS-CoV-2 viral particles (rather than its genomic RNA) in nasal and throat swabs. As shown in Figure 2,[73,78] this sensor is based on a colloidal suspension of AuNPs functionalized with antibodies that target SARS-CoV-2 envelope proteins. When the suspension and the suspected SARS-CoV-2 viral sample are combined, a nanoparticle layer forms on the virion. The resonance peak in the extinction spectrum is shifted as a result of this interaction, resulting in a discernible color change verifying the presence of SARS-CoV-2 [78].

Currently, there are no mutation-specific probes that detect the specific SARS-CoV-2 mutation in a sample. Nanotechnology could provide a solution for this. A simple AuNP lateral flow assay was developed to detect isoniazid-resistant mutations in tubercolosis. The strip could potentially be modified in a short period to design a probe capable of detecting different mutations in SARS-CoV-2 samples [79].

4.2 Nanotechnology in safeguarding

As previously stated, non-pharmacological management has been the key to controlling abrupt outbreaks and ensuring the protection of individuals who are actively involved in a patient's care, including those who handle live samples and those with co-morbidities. Sophisticated and comfortable masks, PPE, and sanitizers thus have a major role to play in this battle.

SARS-CoV-2 viral stability on inanimate surfaces varies depending on the substrate type and texture. It has been found that the residence times of SARS-CoV-2 vary from 4 hours to 7 days, including 4 hours on copper, 4 days on glass, 7 days on plastic, and 2 days on treated wood [80].

Using PPE can prevent the direct contact of the body with SARS-CoV-2 infected matter. A PPE suit contains a face shield that seals the facial area and filters airborne particles,[81] preventing them from entering the respiratory tract. It is essential to have proper disposal mechanisms for used PPE because contaminated textiles can aggravate the spread of the disease. This dumping of biohazardous waste can be solved with self-cleaning/self-sterilizing and hydrophobic materials. The hydrophobic nature of a PPE material can act as a barrier against airborne droplets emitted during coughing or sneezing [70]. The fabrication of reusable PPE kits can be achieved by coating them with self-sterilizing materials. Daylight-active, vitamin K-containing nanofibrous membranes composed of hydrophobic polyacrylonitrile and hydrophilic poly(vinyl alcohol-co-ethylene) have been used for the generation of reactive oxygen species which themselves have bactericidal and virucidal activity. This yields a product which can retain its microbicidal activity even after repeated exposure to bacteria and viruses, confirming it to have effective reusability while also being self-sterilizing. Alternatively, TiO_2-crystal violet nanocomposites and Cu_2O nanoparticle-graphene-based nanocomposites have also shown virucidal activity, demonstrating self-cleaning and hydrophobic characteristics when present in textiles to be used as PPE material.[82] Disinfectants and sanitizers are used to flush microbes from the animate and inanimate surfaces. SHEPROS, a Malaysian firm, has produced a sanitizer product that uses silver nanoparticles with a size of 25 nm and is now on the market. This product can be claimed to be effective against SARS-CoV-2 because it is microbicidal against a wide spectrum of species including viruses. The mechanism of action is known to be affecting the cellular metabolism and cell growth by suppressing the basal metabolism of the electron transport system. Apart from being a potential antibacterial agent, a nonalcoholic aqueous-based colloidal silver solution has also demonstrated antiviral efficacy by inhibiting viral negative-strand RNA production and viral budding.[83] In addition, nanofilms containing $NaClO_2$ crystals which can release disinfectant gas (ClO_2) after UV activation and exposure to moisture, silica/silver nanocomposites, poly(lactic-co-glycolic acid) (PLGA) nanoparticles containing essential oils, alkyl sulphate groups in a cyclodextrin carrier, and tungsten trioxide nanoparticles doped with

palladium nanoparticles also have displayed sufficient virucidal activity for the effective use of those formulations in combating COVID-19 [70].

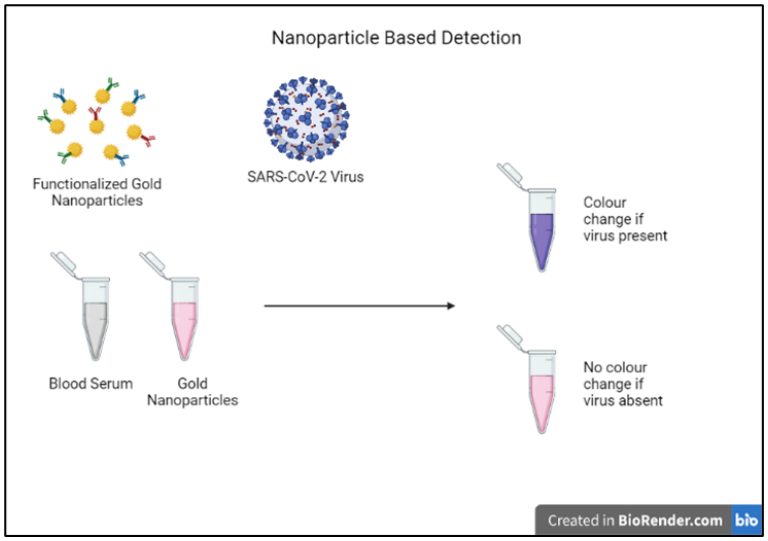

Figure 2. Schematic diagram showing the mechanism by which the colorimetric biosensor detects SARS-CoV-2 using functionalized gold nanoparticles. When the antibodies on the surfaces of gold nanoparticles (colored in red, green and blue) combine with the envelope proteins of the virus, a nanoparticle layer is formed on the virus resulting a visible color change.

One of the main personal preventive precautions during the COVID-19 pandemic period has been to practice wearing a mask. This should ideally involve more than just putting on a mask; the mask should be compatible with the individual and capable of trapping and eradicating SARS-CoV-2 viral particles.[84] A recent study has shown that a sputter-coated silver nanocluster/silicate composite on a mask material has antiviral activity against SARS-CoV-2 .Similarly, the Copper 3D NanoHack mask has been developed by printing apolylactic acid filament on the mask material. It is essential to assemble the mask by hand in the final stage into the three-dimensional form. Especially, all the seams must be manually sealed for an airtight fit.[85] It is obvious that the selection of a fabric for the mask and heat- and light-sensitive nano systems are crucial when developing an active mask to be effective against COVID-19.

A wide range of metal nanoparticles (silver, iron oxide and gold) have shown promising antiviral effects. Antiviral activity of silver nanoparticles (AgNPs) mainly depends on the particle concentration, in addition to the size and shape of the nanoparticles [86]. There are different methods of synthesizing AgNPs that allow the synthesis of particles of varying sizes [87,88]. The virucidal effects of these AgNPs can be employed by incorporating them into PPE and masks. Iron oxide NPs have also demonstrated significant antiviral effects against the H1N1 influenza virus and could be tested for antiviral activity in safeguarding equipment against COVID-19 [89,90].

One way in which antiviral NPs could be implemented into masks or PPE is to combine them with polymers and form (nano)fibrous mats using techniques such as electrospinning [91,92]. There has been significant work performed investigating the potential of electrospun nanofibers as filters and for medical applications [93,94].

4.3 Nanotechnology in the treatment of COVID-19

Nanoparticles have on a number of occasions been examined as a viable alternative to standard antiviral drugs, since the latter have a number of disadvantages such as low bioavailability, hydrophobicity, and narrow spectrum [95]. Nanoparticles small size, large surface area, long half-lives and ability to perform targeted delivery all make them excellent candidates for antiviral treatments. Metal nanoparticles are able to deliver drugs to target viruses and have also been found to have innate antiviral activity [96,97,98]. Polymeric NPs have been investigated as therapies against H1N1 and H5N1 influenza,[99] polio,[100] herpes, HPV and dengue virus [101].

Selenium nanoparticles (SeNPs) have been shown to alter immunological function and diminish the formation of free radicals within host cells in several investigations [102]. Various antiviral medicines, such as ribavirin (RBV) and zanamivir, have been delivered using SeNPs as carriers. RBV has previously shown promise as a treatment for SARS-CoV, MERS-CoV, and most influenza viruses [103]. A recent study of Se@RBV NPs discovered that particles with sizes ranging from 65 to 100 nm inhibited apoptosis caused by H1N1 by blocking the caspase-3 pathway. The particles were found to provide a powerful antiviral effect, and, since COVID-19 patients suffer from chronic inflammation and oxidative stress, this NP therapy could be used to control inflammation and boost their immune response [104].

Furthermore, gene editing via CRISPR/Cas9 technology has also been investigated as a treatment method against COVID-19. CRISPR has the potential to directly target and eliminate the viral RNA and DNA genome which can help in persistent infections [105]. There are concerns about using CRISPR/Cas9 systems *in vivo*, due to their degradation by serum nucleases, unwanted stimulation of the innate immune system and excretion via the kidneys [106]. Nanoparticles have been formulated as carriers for numerous CRISPR/Cas9 systems; Cas9 mRNA from *Streptococcus pyogenes* was co-loaded into an LNP system with modified single-guide RNA and evaluated in an animal model, yielding a stable and biodegradable nanosystem that could edit the transthyretin (Ttr) gene in the mouse liver [107]. In another study, TT3 lipid-like nanomaterials were used to deliver CRISPR/Cas9 to mice to lower HBV DNA expression in the liver [108]. SARS-CoV-2 is a rapidly mutating virus that alters its genetic sequence and thereby its membrane proteins, resulting in reduced effectiveness of vaccines and antibodies, but CRISPR/Cas9 technologies could be used to directly target the genomic RNA and stop it from replicating [109]. Since lipid-like nanomaterials have shown great potential in delivering gene editing CRISPR/Cas9 systems to combat viruses in the liver, lungs and kidneys they could potentially be pursued as a treatment option for COVID-19.

Nanocarriers, including organic-inorganic nanohybrids can also be used as encapsulation vehicles to transport drugs that require selective accumulation, or to reduce systemic side effects of certain antivirals, thereby improving patient compliance [110,111]. Hydroxychloroquine is a drug used in COVID patients to reduce viral load. However, it is responsible for many systemic side effects including retinopathy, myopathy and heart disease. These off-target issues might be mitigated by encapsulating the drug in nanocarriers. Liposomes, polymeric nanoparticles and polymeric micelles (polymersomes) have all been investigated for this purpose [112]. Combination therapy has also been proposed to increase the therapeutic efficacy of individual drugs while reducing the minimum required dose. A lopinavir/ritonavir loaded PLGA NP formulation has been investigated in this regard and demonstrated potent antiviral activity while reducing the required dose.[113]

Therefore, nanomedicine has significant potential advantages in the treatment of COVID-19, including reduced systemic side effects, and the capacity for targeted drug delivery. However, the adverse effects of using nanomedicines should also be considered. Since nanoparticles are manufactured from various different materials, they can cause toxicity at a molecular, cellular and tissue level due to their small size. Tissue level inflammation, generation of reactive oxygen species and disruption of molecular compartments and loss of function are among some of the possible adverse effects [114]. Since most of these studies have been conducted on animals, they are insufficient to determine clinical efficacy and further *in vivo* studies in humans are required, especially to evaluate the effect of NPs on the human immune system.

4.4 Nanotechnology in vaccines for COVID-19

The pandemic's progressively catastrophic effects prompted a massive amount of effort from scientists all across the world, culminating in the world's fastest vaccine development process. Clinical studies of vaccine candidates were conducted and concluded in months, whereas the same process would have taken years previously [115,116]. Surprisingly, the first approved vaccines (Pfizer/BioNTech and Moderna) were the result of novel mRNA-based technology with no previously approved clinical use, rather than traditional vaccine technologies based on inactivated virus particles. Also, significantly most of these vaccine candidates fall in the nanoscale size range, representing the first mass scale implementation of nanoscale vaccines in history [117].

The novel RNA-based vaccines attempt to transfer the genetic code of specific viral proteins to host cells, offering numerous benefits over traditional vaccines that elicit immune responses by injecting whole viruses. Firstly, mRNA is safer than injection of a whole virus since it is not infectious and cannot be incorporated into the host’s genome. In contrast to DNA vaccines, which must reach the nucleus to be decoded, they do not require entry into the nucleus [118]. However, there are challenges in the implementation of this technology, mainly due to the ubiquitous presence of RNA degrading enzymes (RNAses) in the body and the negative charge of mRNA, which makes crossing the cell membrane a challenge [119.120]. As a result, without a functional transport system that allows for the safe translocation of mRNA across the plasma membrane and into the cytosol, it would be unlikely that enough mRNA molecules would be available to deliver the high levels of expression required for immunogenic effectiveness.

To solve this problem, researchers have designed lipid nanoparticle (LNP) based carriers that are positively charged and therefore form stable complexes with mRNA. These are resistant to RNAases and can also facilitate the entry of the mRNA into the cell, where it is translated to antigenic proteins. These are then expelled from the cell and stimulate the antibody production process [119]. The preliminary data from clinical trials of the Pfizer/BioNTech BNT162b2 mRNA vaccine and the Moderna mRNA 1273 vaccine showed efficacies of 95% and 94.5% respectively [8,121]. The US FDA and the European Medicines Agency (EMA) approved these vaccinations for emergency use in late 2020, highlighting their unparalleled level of success with no major side effects [121,122].

It might seem strange that such an effective system could be built within the span of 12 months; however the fact is that lipid vesicles have been researched as drug delivery systems since the 1960s. Liposomes were

the precursors to LNPs and during the past few decades, liposomes have proven to be an extremely versatile nanocarrier platform since they can transport both hydrophilic and hydrophobic compounds references. They have been enormously successful in the clinical setting with numerous liposome formulations used in clinical trials for anticancer, antibiotic, and antifungal drug delivery. They were the first nanoscale drug delivery platform to be translated from the lab to the clinic [123]. Despite their many benefits, liposomes suffer from a number of significant drawbacks, including a short circulation time, lack of selective targeting and *in vivo* instability [124]. Couple this with the fact that the modern pharmaceutical industry is gradually moving away from chemical compounds to the delivery of small molecules and biologics which include complex, specialized therapies such as mRNA, siRNA and DNA molecules that can combat disease at a genetic level, the need for a novel delivery system was sorely felt [125]. LNPs include a cationic lipid that is able to complex with negatively charged genetic material like mRNA and form a stable complex more resistant to enzyme degradation [126].

LNPs have a structure that is similar to that of liposomes, but they differ in that they generate micellar structures within the core that can be modified according to formulation and manufacturing requirements [127]. LNPs typically consist of a cationic ionizable lipid, cholesterol which acts a stabilizing agent, a PEGylated lipid that increases circulation time by masking the LNPs from reticulo-endothelial system (RES) and a phospholipid that encapsulates the lipid structure [128]. The synthesis process of LNPs also offers numerous advantages over conventional systems; use of microfluidics or T-junction mixing enables easy scale up for large scale manufacturing, the cost of raw materials is low, and manufacturing parameters like the components of the lipid matrix, surfactant and emulsifier concentrations, rate of stirring, and temperature can all be easily fine-tuned to obtain the perfect release profile [129,130]. There are certain logistical issues to be addressed such as the thermal instability of the mRNA cargo which means they have to be stored and transported at ultra-low temperatures, resulting in their application in low-income countries being challenging [131]. It is also interesting to keep track of the more conventional vaccine formulations that have gained approval and monitor if the simpler logistical benefits will eventually result in these vaccines gaining prevalence, especially if a booster vaccination protocol is deemed necessary to maintain immunity [132,133,134]. However, the safety and effectiveness of the LNP mRNA vaccines observed during the COVID-19 pandemic will hopefully be translatable to other disease settings, for instance leading to preventive vaccines against influenza viruses, Zika virus and rabies.[118] Providing there are no long-term safety issues associated with the vaccines, caution and unease among patients should subside. This extent of nanotechnology vaccine use that has resulted from the COVID-19 pandemic will undoubtedly have a positive impact on how regulatory bodies, industries, stakeholders and the general public view nanoscience and nanomedicine going into the future.

5. Conclusion

The COVID-19 pandemic has created an unprecedented global health crisis that has affected both developed and developing countries equally. Due to the rapidly mutating nature of the virus, its pathogenicity, and the low availability of vaccines in low-income countries, it has been challenging to obtain herd immunity. Therefore, in the absence of a proven and effective antiviral drug, scientists and physiaions have had to explore the repurposing of various treatments and the implementation of non-pharmacological measures. There have also been many attempts to apply novel technologies and therapies to combat the pandemic. Nanotechnology has attracted particular interest to combat the SARS-CoV-2 virus and overcome the limitations of more conventional methods.

Nanomaterials have been used in many approaches to the fight against COVID-19, the main ones being vaccines, treatment, diagnosis and protection. The mRNA vaccines that were the first approved vaccines on the market helped save thousands of lives but would not have come to fruition so rapidly had there been no nanocarrier to carry the biological. This represents a significant milestone in the history of nanomedicine. This success opens doors for nanocarriers to be used in engineering vaccines for other difficult-to-treat diseases. Nanotechnology has also been used in protection against the virus in the form of disinfectant formulations, textiles and wearable PPE. Furthermore, nanotechnology has also led to the development of new diagnostic biosensors that can detect the virus in real-time at an affordable cost. There is a significant burden on the healthcare sector due to the lack of an efficient tool that can diagnose an infection in real-time, since the average time around the world for an RT-PCR report is 2-5 days. This burden could be reduced considerably by implementing these nanobiosensor diagnostics in a clinical setting. Last but not the least, nanocarriers and other nanoparticle based therapies have been investigated as drug delivery systems to deliver repurposed antivirals and even gene editing platforms like CRISPI/Cas9. These technologies have also shown early promise in the treatment of COVID-19 but further research is required in this area. If there is any takeaway from this global pandemic, it is that humanity was not properly equipped to deal with it, and we must embrace and encourage new technologies and innovations to supplement and eventually replace the conventional treatments that have served us well so far.

Author Contributions

The review was written through the equal contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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