Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

3

Role of nanotechnology for coronavirus detection

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3.1 Introduction

The world has been challenged by many pandemics regularly from the past. Major pandemics such as flu, plague, smallpox, SARS, MARS, EBOLA, etc. have already grievously troubled humanity. The humanity is now facing novel coronavirus disease pandemic, which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It has caused more mortality due to its high infectivity rate, lack of specific treatment, drugs and standard healthcare facilities. On January 30, 2020,

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the novel coronavirus infection was declared as a "public health emergency of international concern" and this disease was named coronavirus disease 2019 (COVID-19).The outbreak of COVID-19 disease was initially noticed in a seafood market in WUHAN city in Hubei province of China in mid-December. The disease has since spread worldwide, leading to an ongoing pandemic. Now it has spread to 221 countries and territories around the world. It was first isolated from three people with pneumonia connected to the cluster of acute respiratory illness. Later, a new strain of coronavirus belongs to the broad family of coronaviruses which was subsequently isolated from bronchialveolar large fluid. The COVID-19 disease is globally resulted in 245,373,039 confirmed cases, including 4,979,421 deaths, reported to World Health Organization (WHO: as of 4:40 pm CEST, October 29, 2021). COVID-19 was proclaimed as pandemic on March 11, 2020 by WHO. It is a deadly infectious disease. The virus mainly transmits from person to person through the droplets from saliva and respiratory secretions of an infected person. Once the virus enters the respiratory tract, it causes upper respiratory infection and subsequently it affects the lower respiratory tract and lungs causing pneumonia [1–5].

The human being can be infected by COVID-19 through different ways. Most infected people would develop mild to moderate illness and recover without hospitalization. The most common symptoms are fever, cough, tiredness, and loss of taste or smell. Less common symptoms are sore throat, head-ache aches, pains, diarrhea, red or irritated eye, etc. As the virus slowly moves toward the bronchial system and lungs, it directly impacts the lungs and damages the alveoli, which leads to organ failure like complications and eventually death. The coronavirus disease is transmitted when people breathe in air contaminated by droplets and small airborne particles containing the virus [6–10]. The COVID-19 disease can also be spread if splashed or sprayed with contaminated fluids in the eyes, nose or mouth and rarely via contaminated surfaces. People remain contagious for up to 20 days and can spread virus even if they do not develop symptoms. The incubation period is ranging from1 to 14 days. The precise interval during which a person with COVID-19 infectious is uncertain. As per current evidence, the period of infectivity starts two days prior to onset of symptoms and declines rapidly within the first week of symptoms onset [11–14]. Currently, there are no effective medication to treat COVID-19. Because clinical manifestation of coronavirus disease ranges from mild flu like symptoms to life threatening pneumonia and acute respiratory illness. It is essential to have proper diagnosis during an early stage of infection for efficient implementation of control measure to slow the spread of COVID-19 [15].

Nanoparticles (NPs) have been widely used in many medical applications, such as biosensing, drug delivery, imaging, and antimicrobial treatment. SARS-CoV-2 is an enveloped virus with particle-like characteristics and a diameter of 60−140 nm. Synthetic NPs can closely mimic the virus

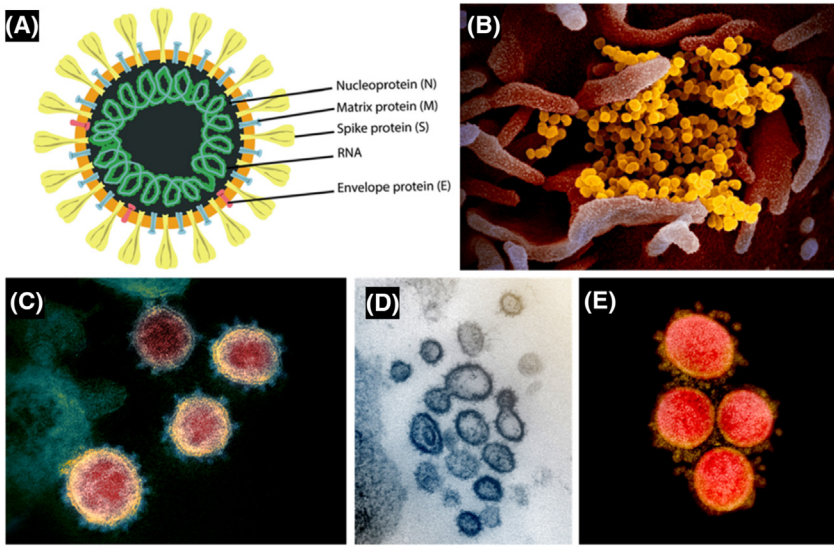

FIGURE 3.1 (A) The SARS-CoV-2 structure is illustrated, with its structural viral proteins indicated. (B) Scanning electron microscopy (SEM) image showing the particulate nature of SARS-CoV-2 (yellow) isolated from a patient in the U.S., emerging from the surface of cells (pink) cultured in the laboratory. Image captured and colorized at NIAID's Rocky Mountain Laboratories (RML) in Hamilton, Montana. Credit: NIAID-RML. (C–E) Transmission electron microscopy (TEM) images show SARS-CoV-2 isolated from a patient in the U.S. Virus particles are shown emerging from the surface of cells cultured in the laboratory. The crown-like spikes on the outer edge of the virus particles give coronaviruses their name [21].

and interact strongly with its proteins due to their morphological similarities. Hence, NP-based strategies for tackling this virus have immense potential. NPs have been previously found to be effective tools against many viruses, especially against those from the *Corona viridae* family [16].

3.2 Structure of COVID-19

SARS-CoV-2 is an enveloped single-stranded positive RNA-virus with a large RNA genome of approximately 30kb with genome characteristics similar to the corona virus (Fig. 3.1). On the basis of genomic organization and phylogenetic relationship, coronaviruses have been classified into subfamily, that is, Coronavirinae that consists of four genera such as Alpha coronavirus, Beta coronavirus, Gamma coronavirus, and delta coronavirus. The coronavirus genomic RNA encodes replication and transcription complexes from a single large open reading frame and structural proteins of virus. The major structural proteins of coronavirus are spike, envelope, membrane, and nucleocapsid.

(a) Spike(S) protein: The spike protein is a highly glycosylated and large type I transmembrane fusion protein that is made up of 1160 to

1400 amino acids depending upon the type of virus. As compared to other proteins that are primarily involved in virus assembly, the spike protein plays a crucial role in penetrating host cells and initiating infection.

(b) Envelope protein-: Envelope protein is contributed to virus assembly and as a membrane protein, also possesses viroporin channel properties that may contribute to epithelial barrier damage, pathogenesis, and disease severity.

(c) Membrane protein: This may contain a T-cell epitope cluster revealed by the immunogenic and structural analysis of a panel of truncated peptides overlapping with Mn2 and Md3. The M-protein of SARS-CoV-2 holds dominant cellular immunogenicity.

(d) Nucleocapsid protein-: N proteins are expressed abundantly during infection. High levels of IgG antibodies against N protein have been detected in sera from SARS patients. The N protein is a representative antigen for the T cell response in a vaccine setting [2].

3.3 Nanotechnology toward the diagnosis of COVID-19

For combating the COVID-19 disease, nanotechnology-based approach should be initiated. This technique can be employed in number of ways toward the betterment of mankind, which includes the development of drugs and vaccine and high responsive testing kits for detecting the infections or immunity. This technique can also be used in developing super fine filters face mask through surface coatings. It will offer higher opposition to the adhesion of virus by inactivating the toxic nature and will improve the tools for contact tracing [17].

Using metallic gold nanoparticles, a colorimetric assay was developed by Moitra et al., through which naked eye detection of SARS-CoV-2 virus was performed. Gold nanoparticles (AuNPs) were capped suitably designed thiol-modified antisense oligonucleotides (ASOs) which is specific for N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2. This experiment resulted with the diagnosis of positive COVID 19 within 10 minutes. Fig. 3.2 shows the schematic representation showing the detail procedure and techniques considered toward the detection of SARS-CoV2 virus. The process includes the collection of viral sample and extraction of viral and then RNA was added to the ASO-capped gold nanoparticles. The thiolated ASO-capped AuNPs are found to be agglomerated and it is observed after five minutes incubation. This agglomeration is because of the target RNA sequence of SARS-CoV-2 which exhibits a change on its surface plasmon resonance. Again, RNaseH was added and incubated for 5 min and at a temperature of 65°C. Finally, precipitation of gold nanoparticles was visually detected [18].

Again, a dual functional plasmonic biosensor was developed by Qiu et al. for rapid clinical diagnosis of COVID-19. The combine behavior

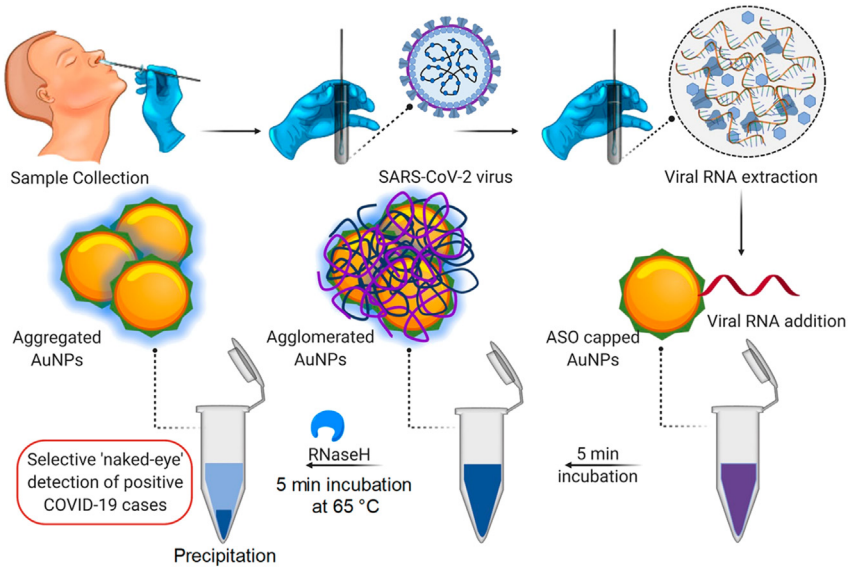

FIGURE 3.2 Schematic representation for the selective naked-eye detection of SARS-CoV-2 RNA mediated by the suitably designed ASO-capped AuNPs. This figure was adopted from Moitra et al. [18].

of plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR), make the biosensor more effective. It uses two-dimensional nanoislands of gold nanoparticles which is functionalized with complementary DNA receptors. Through nucleic acid hybridization, the SARS-CoV-2 is detected. The biosensor exhibits a high sensitivity with a limit of detection of 0.22 pM toward the selected SARS-CoV-2 sequences [19].

Abo-zeit et al. have performed a molecular docking study repurposes US food and drug administration (FDA)-approved iron oxide nanoparticles for combating and controlling the COVID-19 infections. Prior to this iron oxide nanoparticles have been approved by the FDA for curing the anemia treatment. Several studies related to the in vitro antiviral activities have been demonstrated. The docking study was performed to explore the interaction of IONPs (Fe_2O_3 and Fe_3O_4) with the spike protein receptor binding domain (S1-RBD) of SARS-CoV2 that is required for virus attachment to the host cell receptors. These interactions of IONPs are expected to be associated with viral proteins conformational changes and hence, viral inactivation. Therefore, we recommend FDA-approved-IONPs to proceed for COVID-19 treatment clinical trials [20].

Shan et al. have developed a nanomaterial-based sensor array by linking different gold nanoparticles to organic ligands, which has the ability to detect the COVID-19 from the exhaled breath. The array of gold nanoparticles provides excellent sensing layer that can swell and shrink upon exposure to volatile organic compounds (Fig. 3.3). It causes change in

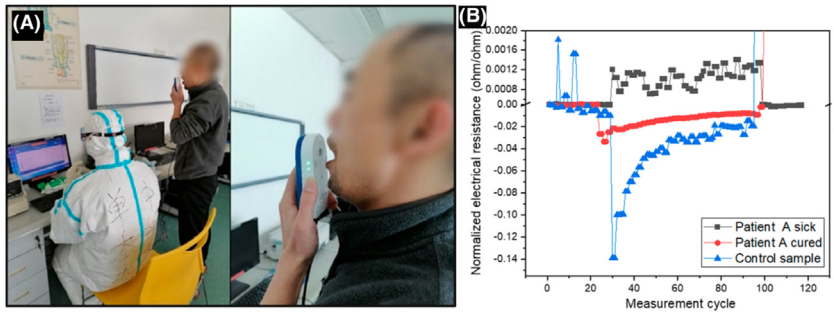

FIGURE 3.3 (A) Example of breath collection with the developed hand-held breathalyzer system from a patient in Wuhan, China. (B) Representative response of a sensor to three different breath samples. The normalized response of sensor 7 of the breathalyzer system to three different samples: patient A, COVID-19, first sample while infected; patient A, second sample after determined as recovered; and a healthy control. The x-axis represents the cycle measurement; each unit is one cycle of the sensor. The infected sample had a positive change response, while the recovered and control showed negative charges [16].

electrical resistance which leads to sensing. They have conducted the study by observing 49 confirmed COVID-19 patients, 58 healthy controls, and 33 non-COVID lung infection controls [16].

An array consisting of eight gold nanoparticles working on the principle described earlier in the text was developed and integrated with electronic circuitry and an advanced apparatus that collects an exhaled breath sample, by blowing into the device for 2−3 s from a distance of 1−2 cm. A built-in sensor technology advised the study subject when the test was complete. As the breath passes through the array, a mixture of COVID-19-related VOCs reacts with the sensors, and these emit a set of electrical resistance signals as a function of time. An example of a sensor response to different samples can be found. If the breath collection was not satisfactory, the subject was asked to repeat the test. Software-based machine learning methods probe the pattern of output signals to get a signature of COVID-19. A more detailed description is provided in the Materials and Methods section, the Supporting Information using this nanotechnology, an exploratory clinical study in Wuhan, China, was conducted during March 2020 on 140 participants. The selection of participants from three distinct groups COVID-19 patients, healthy controls, and non-COVID lung infection/disease controls is described. The characteristics of the 140 participants are tested. Among the patients, more than 60% had no underlying chronic disease, while all the rest suffered at least from hypertension; 10% of those suffered from diabetes mellitus. Most patients were nonsmokers (73%), with a mean age of 59 years, with 57% females. For the control group, 67% were nonsmokers, and the mean age was 52 years, with 46% females. For the lung infection

control group, 73% were nonsmokers, and the mean age was 63 years, with 44% females. Statistical analysis was employed on the signals emitted from three binary comparisons: COVID-19 vs. control; COVID-19 vs. other lung infections; and COVID-19 first vs. COVID-19 second sample. Seventy percent of the data were used to calculate the discriminant factor analysis (DFA) models. For each comparison, a receiver-operating-characteristic (ROC) curve and cut-off value were determined. The remaining test data were classified blindly based on the cut-off as presented. Panels A, B, and C show data classification from cumulative sensor responses to breath samples as represented by the canonical variable of the discriminant analysis. Panel D shows ROC curves for the cumulative breath-sensor response. The results showed an area under the curve (AUC) of 0.81 (95% CI, 0.70–0.89) in patients with COVID-19 vs. with controls, 0.97 (95% CI, 0.92–0.99) in COVID-19 vs. other lung infection/conditions, and 0.87 (95% CI, 0.671.00) in COVID-19 first sample vs. COVID-19 second sample. $P < .001$ was found for the comparisons of the training set for each of the binary classifications.

Lew et al. have developed a colorimetric serological assay by conjugating short antigenic epitopes to gold nanoparticles, for detecting the SARS-CoV-2 IgGs in patients' plasma. In SARS-CoV-2, four immunodominant linear B-cell epitopes are present on the spike (S) and nucleocapsid (N) proteins. These epitopes were characterized owing to their IgG binding affinity and they can be used as highly specific biological motifs on the nanoparticle to identify the target antibodies. The binding affinity and the specificity of the four epitopes were determined by using two distinct techniques, that is, surface plasmon resonance (SPR) and fluorescence polarization (FP) techniques (Fig. 3.4A and B). SPR spectroscopy detects binding between immobilized ligands and target analytes at the surface of a thin metal film via changes in the refractive index. SPR sensorgram data confirmed the binding of all four epitopes when titrated against varying concentrations of epitope-specific SARS-CoV-2 IgGs (Fig. 3.4C). For determining the selectivity of these epitopes, an orthogonal FP approach was also pursued and further it confirms their IgG binding affinity. FP detects the binding of a fluorescent ligand (FITC-labeled epitopes, in this case) to a larger molecule (IgG) by measuring the increase in the polarization of fluorescence emission induced by the formation of a larger complex. Compared to SPR spectroscopy, FP is a free solution technique that allows for high-throughput screening and does not require any separation of bound and free ligand. The three S-protein-based epitopes (S14P5, S20P2, and S21P2) exhibited a significant increase in FP values upon the introduction of SARS-CoV-2 IgGs relative to the addition of the closely related SARS-CoVIgGs or the control normal human IgG (Fig. 3.4D).

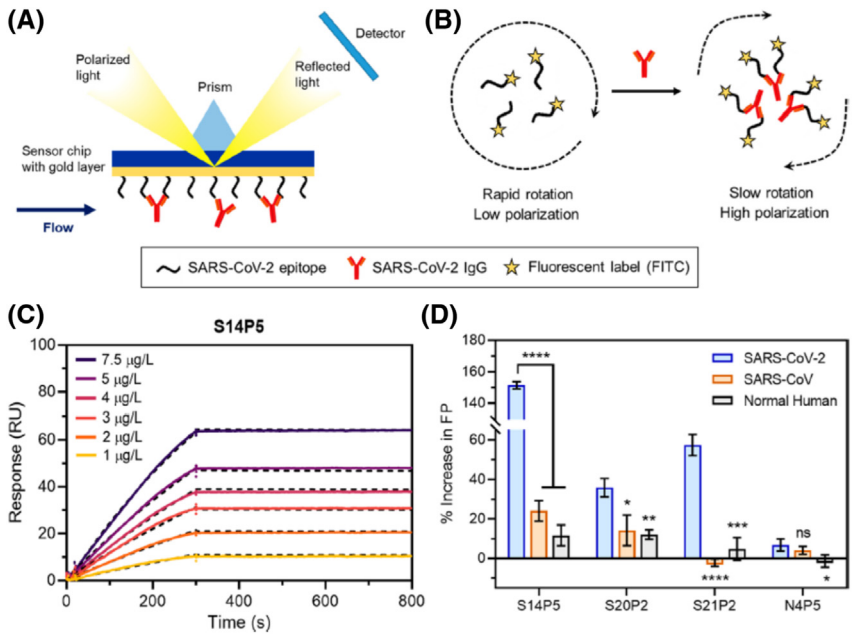

FIGURE 3.4 Characterization of the binding affinity and specificity of four SARS-CoV-2 linear B-cell epitopes. Schematic depiction of (A) SPR spectroscopy and (B) FP working principles to characterize the epitopes' binding affinity and specificity toward SARS-CoV-2 IgG. (C) SPR sensorgram data showing real-time binding of anti-SARS-CoV-2 S1 IgG at varying concentrations to the immobilized S14P5 epitope. Dashed line represents model fit. (D) Relative FP increase when 100 nM peptide epitopes were treated with 200 nM SARS-CoV-2, SARS-CoV, and normal human IgGs [12].

Conclusion

Herein, we have summarized the role of nanotechnology for the COVID treatment. The various detection methods have been discussed in detail. For preventing the COVID-19, nanotechnology-based methodology should be instigated. This method can be utilized in number of ways toward the advancement of manhood, which involves the growth of drugs and vaccine and high responsive assessment kits for detecting the diseases. Although, number of steps have been made, but still, we need to work hard and proceed to use the nanoapproach for future real time application. We need to identify the challenges and need to work on them on a stepwise manner. There is no hurry, but a continuous work should keep as the forefront. Hope everything will be solved and once again nanotechnology will be our savior.

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Non-Print Items

Abstract

The Coronavirus disease 2019 (COVID-19) is the worst pandemic faced by the mankind in current millennium. It is due to the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This was detected for the first time in December 2019 with rapid human-to-human transmission. The global pandemic has spread out across 213 countries by affecting millions including 52.7 lakh death. Currently, there are no clinically proven therapeutic methods that clearly inhibit the effects of this virus. Nanoparticles (NPs) have been widely used in many medical applications, such as biosensing, drug delivery, imaging, and antimicrobial treatment. Looking the rapid, cost effective and accurate sensing of the virus, the field of nanoscience and nanotechnology is working actively. By following the colorimetric method and surface plasmon spectroscopy, several nanoparticles are used in quantifying and detecting the COVID-19. This chapter contains the rapid analysis and quantification of COVID-19 by the arrangements of several nanoparticles including gold nanoparticles, iron oxide nanoparticles, etc.

Keywords

Coronavirus disease; Nanoparticles; Sensing; Diagnostic devices; Healthcare management; Translational research