ACS NANO

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REVIEW

Opportunities and Challenges for Biosensors and Nanoscale Analytical Tools for Pandemics: COVID-19

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Cite This: *ACS Nano* 2020, 14, 7783–7807

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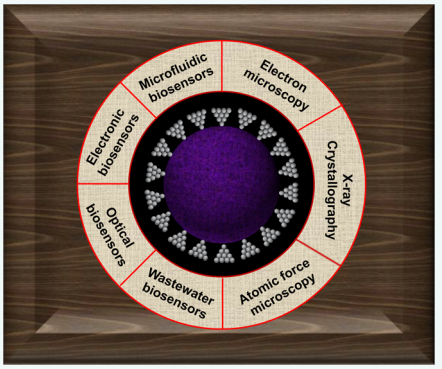
ABSTRACT: **Biosensors and nanoscale analytical tools have shown huge growth in literature in the past 20 years, with a large number of reports on the topic of 'ultrasensitive', 'cost-effective', and 'early detection' tools with a potential of 'mass-production' cited on the web of science. Yet none of these tools are commercially available in the market or practically viable for mass production and use in pandemic diseases such as coronavirus disease 2019 (COVID-19). In this context, we review the technological challenges and opportunities of current bio/chemical sensors and analytical tools by critically analyzing the bottlenecks which have hindered the implementation of advanced sensing technologies in pandemic diseases. We also describe in brief COVID-19 by comparing it with other pandemic strains such as that of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) for the identification of features that enable biosensing. Moreover, we discuss visualization and characterization tools that can potentially be used not only for sensing applications but also to assist in speeding up the drug discovery and vaccine development process. Furthermore, we discuss the emerging monitoring mechanism, namely wastewater-based epidemiology, for early warning of the outbreak, focusing on sensors for rapid and on-site analysis of SARS-CoV2 in sewage. To conclude, we provide holistic insights into challenges associated with the quick translation of sensing technologies, policies, ethical issues, technology adoption, and an overall outlook of the role of the sensing technologies in pandemics.**



KEYWORDS: *nanosensors, nanoplasmonics, sewage sensors, microfluidics, atomic force microscopy, X-ray diffraction, electron microscopy, point-of-care-technologies, COVID-19, pandemics*

In the last 15 years, humans have witnessed 5 pandemic diseases, severe acute respiratory syndrome (SARS), Swine flu, Ebola, Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19), where both MERS and COVID-19 are actively present within our community, with the latter having more severe complications and infection rates in recent times. COVID-19 is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) virus, which was actively revealed in Wuhan, China in December 2019 and later sequenced in January 2020.[1,2] In just a period of 2 months (March 2020), the disease had spread to 90% of the countries on our planet. In parallel, there remains a great interest in the biosensors and nanoscale visualization/characterization tools such as the electron microscopy (EM), atomic force microscopy (AFM), and X-ray diffraction (XRD) for the early detection and revealing prognostic features of a given pathogen.[3,4] Recent advances have also extended the use of these advanced sensing techniques into the development of high-throughput devices capable of screening a large number of candidate compounds for quick drug discovery and vaccine development during pandemics.[5−7] Most of the latest sensors currently available in both academia and industry are based on the electrical or optical transduction methods.[8] These include single-molecule sensors,[9] wearable devices,[10] ingestible capsules,[11] disposable screen-printed electrodes,[12] and hand-held portable instruments[13] which could actively detect the infectious disease-causing pathogen at an early stage of the infection. Nevertheless, despite their vast availability and well-

Received: May 27, 2020
Accepted: June 18, 2020
Published: June 18, 2020

https://dx.doi.org/10.1021/acsnano.0c04421
ACS Nano 2020, 14, 7783–7807

Table 1. Categorization of Biosensing Strategies and Surface Characterization Techniques for Virus Detection

		mechanism	applications			
			diagnosis	prognosis	drug discovery	drug analysis
direct detection of virus		complete virus is detected individually using biosensors or more commonly by Petri dish-based cell culture techniques[18,19]	X	X	–	–
viral RNA/DNA detection		RT-PCR, PCR principles applied either in conventional nucleic acid formats (with fluorescence or radioactive labels) or using advanced techniques such as LSPR, SPR, QCM, and other potentiometric sensing techniques[20−22]	X	X	X	–
antibody/antigen detection		microwell-based bioassays using absorbance readers and several optical and electronic biosensors which essentially measure the binding kinetics of the biomolecule of interest[23]	–	X	X	X
surface characterization tools as complementary sensing techniques	AFM	high-resolution scanning probe microscopy with a resolution 1000 times smaller than the optical diffraction limit used for investigating surface properties of viruses in the order of fraction of a nanometer[24,25]	–	X	X	X
	EM	the surface of a virus is imaged with a focused beam of electrons to identify topographical features[26]	–	X	X	X
	XRD	crystallographic features of virus are mapped to determine 3D structures of the viruses[27,28]	–	–	X	X

[a]'X' denotes suitability of application and '–' indicates application not applicable. In the application column, we refer to diagnosis as a quick detection either for in use in hospital or self-testing. Note that visualization tools can still be used for diagnosis but are deemed unsuitable for quick detection.

known advantages in academia, the full potential of the biosensing and characterization tools is yet to be harnessed in on-site applications, such as during the outbreak of an infectious disease. Certainly, there are scientific challenges and opportunities for bio/chemical sensors before they can live up to the expectation of accurate, precise, and early diagnosis of emerging diseases. It is in this context that we present an analysis of the state of art instruments and technologies, in particular concerning COVID-19, for the detection and analysis of pandemic strains. In general, we can segregate the detection of viruses into three main categories: (1) direct detection of the virus, (2) viral RNA/DNA detection, and (3) antibody detection.

These sensing strategies are further complemented by the aforementioned surface visualization/characterization tools (AFM, EM, XRD) presenting a complete package of technologies that can potentially be used for diagnosis, prognosis, drug discovery, drug analysis, and disease spread surveillance, all of which are vital for ensuring good community health.[14,15] A general overview of these groups including their mechanisms is discussed in Table 1. It is also obvious that for quick technology development, such as for the development of biosensors for COVID-19, it is essential to build on the existing scientific evidence for the use of technology in similar applications. Therefore, we begin by highlighting similarities and differences between other RNA viral infections from the perspective of understanding the COVID-19 disease and subsequent technology development to serve the required demand for sensing and characterization of the SARS-CoV2 virus.

SIMILARITY OF SARS-COV-2 WITH OTHER VIRAL STRAINS

COVID-19 disease is highly contiguous as its transmission is encouraged by airborne droplets and touch-based human to human contact.[16] Common symptoms of this disease are similar to influenza, and therefore, it is not trivial to rapidly detect the disease accurately. Comparative analysis of the SARS-CoV2 with other epidemic viral strains serves as background information on which researchers can build upon to create potential technologies to quickly address the demands of disease control in an emergency.[15] While there are several similarities and differences between these viruses, here we focus only on those features which will help researchers working in the field of biosensors and surface visualization/characterization to develop strategies for accurate detection and morphological analysis of SARS-CoV2. One striking similarity between SARS-CoV2 virus and influenza, H1NI, SARS-CoV, and MERS viruses is that all contain RNA as their genetic material, therefore also known as RNA viruses. Compared to DNA viruses, RNA viruses are technically more virulent as they infect cells by injecting RNA which quickly transcribes and replicates viral proteins in the host cell.[17] This also makes it extremely challenging to detect a RNA virus at an early stage of the infection. Cell culture and nucleic acid-based tests have been the gold standard since their inception as compared to all other techniques.[18]

However, unlike the DNA viruses which have polymerase machinery, the nucleic acid tests for RNA viruses include additional steps of reverse translation of RNA to DNA before it is amplified.[30] Structurally, as viruses responsible for COVID-19, SARS, and MERS outbreaks belong to the same category of beta coronavirus family, these viruses are mostly spherical, but sometimes pleomorphic in shape.[31,32] The average diameter of these viruses is 125 nm, where their capsid covering the genetic material is around 85 nm and glycoprotein spikes on the surface of the capsid are roughly 20 nm;[33] see more out of scale structural features of SARS-CoV2 virus in Figure 1. Comparing genetic characteristics of the SARS-CoV2 virus with other viruses may also provide useful insights to develop sensing strategies for the early detection of COVID-19. For example, untranslated regions (UTR) of the RNA, both at 5 prime and 3 prime ends are involved in the binding of the cellular proteins.[34] The detailed arrangement of coding regions of coronaviruses is given in Figure 2. In addition, the mechanism of how coronaviruses infect cells can also be useful for developing sensors for analyzing the disease mechanism and drug discovery. In brief, the SARS-CoV2 virus enters a given cell *via* protein−protein interactions where the glycoprotein spikes bind to the angiotensin-converting enzyme 2 (ACE2) on the cell surface.[34] This attachment of the virus on the cell and further entry into the cell is assisted by protease enzyme called TMPRSS2. After the entry of the SARS-CoV2 virus into the cells, the RNA quickly translates

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ACS Nano 2020, 14, 7783−7807

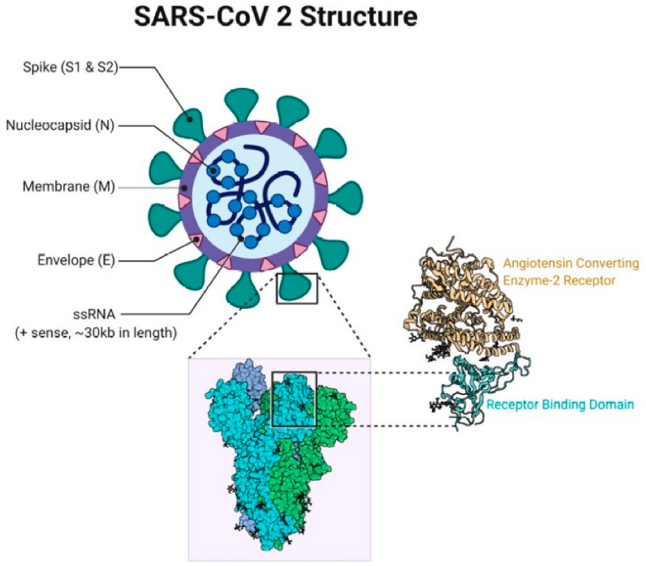

Figure 1. Structural view of SARS-CoV2 virus and its surface protein. Reproduced with permission from ref 29 under a Creative Commons CC-BY license. Copyright 2020 StatPearls Publishing.

into proteins, including the RNA synthesis in the cytoplasm by viral replication.[31]

CURRENT DETECTION AND TRACING TECHNOLOGIES FOR COVID-19

Current detection for COVID-19 is primarily based on the combination of two or more techniques which include RT-PCR, chest X-ray, computed tomography (CT) scans, and the detection of some common biomarkers in the blood.[35] These biomarker tests include identification of elevated levels of the C-reactive protein, low procalcitonin, low lymphocyte counts, and high concentration of interleukin 6 and interleukin 10. Details of reverse transcriptase polymerase chain reaction (RT-PCR), CT scans, biochemical assays, and the development of mobile phone-based digital contact tracing applications are discussed in the proceeding subsections.

Molecular Method. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) is a method used to detect the presence of nucleic acid-based genetic sequences from any organism, including viruses.[36,37] To perform RT-qPCR for SARS-CoV2, first the biological fluid where the virus strains are present, upper and lower respiratory fluid, is collected. The collection of fluid is generally performed using nasopharyngeal and oropharyngeal swabs.[38] Then the collected fluid undergoes a number of filtration and separation steps to isolate the viral RNA. Using the reverse transcriptase enzyme, complementary viral DNA (cDNA) is generated from the viral RNA. Specific regions of cDNA then undergo a polymerase chain reaction for amplification, where an additional DNA probe, designed to hybridize within a small part of the specific region of cDNA, is incorporated to enable real-time detection of the amplification process. Traditionally, radioactive isotopes were used as markers to target the specific nucleic acids, but more recently, fluorescence tags (fluorophore and a quencher) are used on the DNA probe for the real-time detection.[39] Essentially when the DNA polymerase enzyme is adding nucleotides to the specific part of the viral cDNA, it encounters the double-stranded DNA in its path (due to DNA probe) where the exonuclease activity of the polymerase enzyme separates the fluorophore and the quencher molecules to produce real-time detection of the viral cDNA. If the number of cDNA copies (proportional to the concentration of the virus) produced after transcription is high, a large amount of fluorescence signal is generated after few rounds of polymerase reaction, and if the system is calibrated well, the fluorescence intensity is directly proportional to the concentration of virus in the infected patients. The typical sensitivity that can be achieved by RT-PCR is between 500 and 1000 copies/mL of viral RNA. Among the current tests, three regions of the cDNA have been identified for the detection of the SARS-CoV2 virus, which are the *Rd*RP, *E*, and N genes. More details about these sequences are described in Table 1 of the work from Udugama *et al.*[38] and on the Center for Disease Control and Prevention website.[40] One challenge that remains is also that SARS-CoV2 damages the target RNA when opening its viral capsid. One reason might be due to the immune response of the host body. This leads to a release of small fragments of RNA into the bloodstream which is challenging for detection by RT-PCR. Perhaps, signal enrichment strategies for isolation of RNA fragments using advanced technologies such as CRISPR or in combination with nanomaterials such as gold nanoparticles and metal−organic complexes may provide a solution to this problem. Another reason for poor sensitivity using RT-PCR is that the RNA easily gets degraded and typically requires an

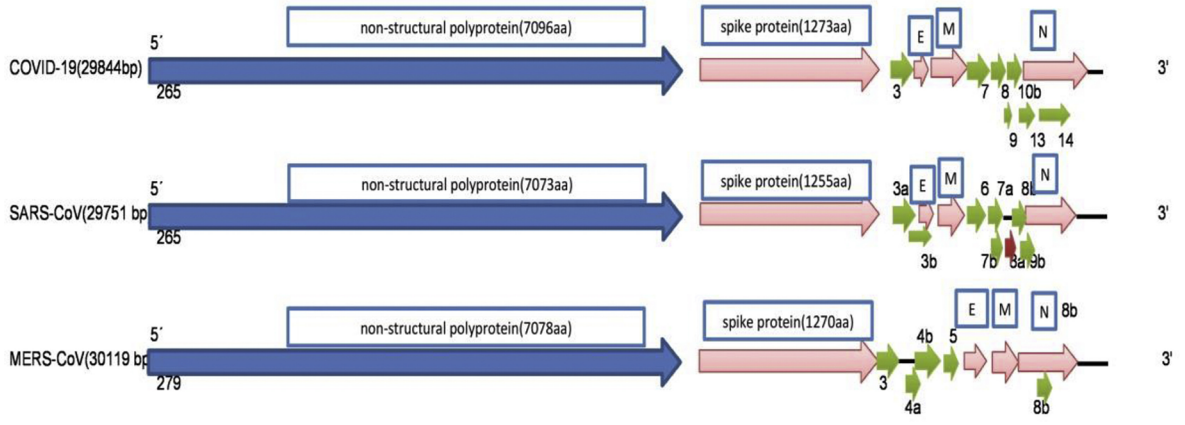

Figure 2. 5′ UTR and 3′ UTR and coding region of COVID-19, SARS-CoV and MERS-CoV. Reprinted with permission from ref 34. Copyright 2020 Elsevier.

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ACS Nano 2020, 14, 7783−7807

immediate frozen storage condition, and the completion of handling samples may also induce the poor detection. However, the point-of-use sensors, discussed later, may provide an immediate testing of RNA and will overcome this issue from the samples.

Computer Tomography. Computer tomography, which is more popular with the name of CT scan, is being used either in combination or as a standalone diagnostic tool to confirm the false hits from RT-PCR for the detection of SARS-CoV2 virus.[41−43] In this method, usually a large number of X-ray measurements of the chest are taken from different angles to generate three-dimensional (3D) images with contrast, which are later analyzed by radiologists for abnormal features to authenticate the presence of SARS-CoV2 infection. Signatures of COVID-19 infection which appear in CT scan include areas of subpleural regions of ground glass opacification affecting the lower parts of either a single lobe or both lobes.[44] In addition, data from COVID-19 patients have shown consolidation of fluids in the lungs. Moreover, the CT images have so far also assisted in serving as a prognostic tool for COVID-19. For example, in the early days (0−2 days) of the infection, the CT scan resembles more of a normal chest condition. As the disease progresses, the opacity of the scan increases, for instance, patients after 4 days of the infection have been reported with ground glass opacity which even appears as irregular patterns in the scan. Figure 3[45] shows a CT scan

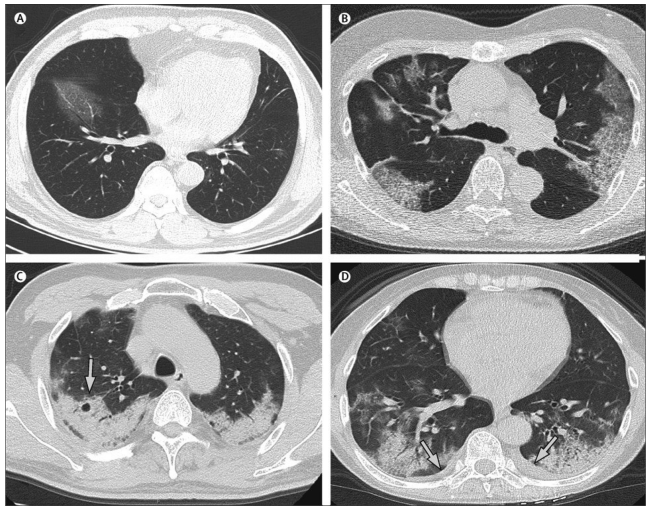

Figure 3. Transverse thin-section CT scans in patients with COVID-19 disease: (A) 56 year-old man, day 3 after symptom onset; (B) 74 year-old woman, day 10 after symptom onset; (C) 61 year-old woman, day 20 after symptom onset; and (D) 63 year-old woman, day 17 after symptom onset. Reprinted with permission from ref 45. Copyright 2020 Elsevier.

image from different patients on different days where the various abnormalities are clearly visible such as focal ground glass opacity and include bilateral and peripheral predominant consolidation after 20 days of the onset of the disease. While CT scans are likely to remain as one of the most important tools in the early diagnosis of the COVID-19, the major challenge for the radiologists lies in the distinction of symptoms from other lung disorders or pneumonia-like symptoms which are not due to COVID-19 infections. In fact, the main caveat of using CT scans for COVID-19 is that the specificity of the detection is around 25%, as reported in the literature so far,[44,46] as the imaging features overlap with other viral pneumonia. In addition, CT scans are expensive and require advanced technical skills for the operation and analysis, and it can only be adapted as a complementary technology with RT-PCR for SARS-CoV2 detection.

Biochemical Tests. Biochemical tests such as the enzyme-linked immunosorbent assay (ELISA) have also been used to detect the viral protein or the antibodies that are created by our body in response to a SARS-CoV2 infection for the diagnosis of COVID-19 disease. The standard procedure to detect these antibodies involves the use of microtiter plates such as the 96-well, where antibodies are detected *via* protein−protein interactions. These interactions are amplified using a fluorescence, luminescence, or colorimetric type of detection assisted by enzymes involved in the reaction. However, one main issue is that as the amount of viral load changes during the course of the infection, so detecting low concentrations of viral protein might be difficult. For instance, To *et al.*[47] showed salivary viral loads in the first week after the onset of symptoms, which gradually decreased with time. On the other hand, detecting antibodies generated in response to the viral infection is more specific for confirming presence of the COVID-19 disease. Nevertheless, there are still potential challenges with developing accurate serological tests such as those related with the cross-reactivity of SARS-CoV2 antibodies with other antibodies generated against coronaviruses.[48] There are still some important advantages of the antibody testing, for instance, the presence of antibodies can verify if vaccines are functioning as intended.[49] It can also be used in disease contact tracing applications, weeks after an individual is infected with the virus. Perhaps currently it is the most important test to help inform the intervention policymakers on how many asymptomatic cases exist in a given population.[50] However, false negative results from the current antibody tests are often reported which are attributed to the combination of following technical reasons: (1) a low concentration of antibodies typically present in fluidic samples; (2) presence of homologous proteins; and (3) lack of sensitivity from the detection instrument. While, low concentration of antibodies and the presence of homologous proteins will remain challenging for the biosensing community, the sensitivity of the current tools could perhaps be enhanced by development of an engineered protein which will attach specifically to the antibody of interest. However, the ultimate solution is the development of ultrasensitive and selective biosensors, which we discuss more in the proceeding sections.

Contact Tracing. Use of digital technologies, especially smartphone-based applications to identify the presence of infected cases, both active and recovered, has also been implemented successfully to manage the spread of COVID-19.[51] Ferrite *et al.*[52] recently developed an application for contact tracing, see more in Figure 4. In this application, the main idea is to replace a manual contact tracing with instantaneous transmission of signals to and from a central server. Essentially, the SARS-CoV2 virus cases are directly communicated to the server, which enables recommendation of risk-stratified quarantine and social distancing measures in those now known to be possible contacts. This is done by preserving absolute anonymity of the infected person. In addition, tests can also be requested by symptomatic individuals through the developed application. One of the pros of such software-based technology is that it can easily be tuned to be more informative, for example, quarantining areas

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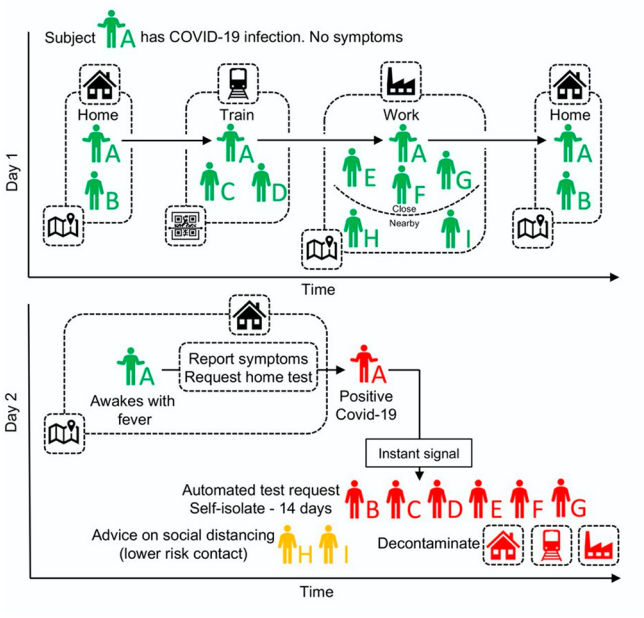

Figure 4. Contact tracing application: Using GPS contacts of individual A and all individuals using the app, infections are traced out. This is further supplemented by scanning QR-codes displayed on high-traffic public amenities where GPS is too coarse. Using this application individual A requests a test for COVID-19 infection, and their positive test result is shared as an instant notification to individuals who have been in close contact. Reproduced with permission under a Creative Commons CC-BY license from ref 52. Copyright 2020 American Association for the Advancement of Science.

if the disease spread in that area becomes uncontrolled, quarantining specific flats or families, or performing second or third degree contact tracing if number of cases rise. Another advantage of contact tracing is the accessibility of technology with members of the community. In the past few years there has been a steady rise in the adoption of smartphones and mobile internet which could potentially facilitate a coordinated response during pandemics. One main limitation of contact tracing is that to harness the full potential of the technology, it is also necessary to increase the number of diagnostic tests. Another limitation such as privacy consideration also exists, which with changing time and circumstances are becoming more acceptable within the community members.[53] The use of contact tracing however is still useful for members of a community to track the movement of people who came in contact with the infected, including the asymptomatic ones.

CHARACTERISTICS OF COVID-19 DISEASE FOR DEVELOPING STRATEGIES FOR ANALYSIS

Physical Characteristics of SARS-CoV2 Virus. There are four major structural proteins in SARS-CoV2 virus: E (envelope protein), M (membrane protein), N (nucleocapsid protein), and S (spike protein).[54,55] Each protein not only individually plays a role in the virus structure but also is involved in the mechanism of the replication cycle.[56] The smallest and most enigmatic major proteins of CoV viruses is the E protein. During the replication cycle, although a small portion of E protein is incorporated into the virion envelope, it is abundantly expressed inside the infected cell.[57] The primary function of the N protein is to bind to the CoV RNA genome and to form the structure of the nucleocapsid.[55] In addition, of its major role in processes of viral genome, it involves the CoV replication cycle and also the host cellular response to viral infection.[58] The shape of the viral envelop is defined by the M protein.[59] The other function of M protein is its role as the central organizer in CoV assembly through interactions with other major structural proteins.[60] Coronavirus enters into host cells by the attachment of transmembrane spike (S) glycoprotein to the host surface receptor. Also, the S protein mediates the subsequent fusion between the viral and host cell membranes to make the entry of viral cell easier into the host cell.[57,61] The S protein binds to ACE2, a transmembrane receptor which is widely expressed in the lungs, kidneys, gastrointestinal tissue, and heart.[62] The binding to the host cell receptor and fusion between viral and cellular membranes have been performed with two subunits of the S protein: S1 subunit and S2 subunit, respectively.[63] The S2 subunit contains neutralizing epitopes of the virus, including a conserved fusion peptide, a transmembrane domain, a cytoplasmic domain, and heptad repeats 1 and 2.[64] In the S1 subunit, the core receptor binding domains are highly conserved. The differences in the amino acids are the cause of the direct interaction between spike protein and host cell receptor.[65]

Potential Small Molecule Biomarkers in Blood. A range of small molecule biomarkers in the blood has recently been identified for the COVID-19 detection from patients all around the globe. While many of these biomarkers are not as specific as detecting the viral RNA or DNA directly from the blood or by using RT-PCR, their concentration in the blood is found to be useful for the prognostics of the COVID-19 disease. This is due to the fact that many protein-based molecules in the blood, which eventually serve as the biomarkers of a disease, increase or decrease their levels in order to fight against the infection. Therefore, some of these biomarkers are directly linked to the severity of the infection. Particularly, serum urea, (CREA), C (CysC) serum direct bilirubin (DBIL), (CHE), and lactate dehydrogenase (LDH) concentrations were found to be significantly higher in severe COVID-19 patients than those in mild COVID-19 patients.[66−68] More recently, smell dysfunction has been proposed as a biomarker for the COVID-19 disease.[69] The mucous secretions from the nose contain many protein molecules that help metabolize xenobiotics and support epithelial integrity required for smell function. Therefore, developing biosensors for the detection of mucous proteins may also be useful for the early detection of the SARS-CoV2 infection. However, it should be noted that these biomarkers may not be specific to COVID-19 as nasal dysfunction is reported in other diseases such as the Parkinson's disorder.[70] More qualitative and quantitative information regarding the trend of biomarkers for COVID-19 is summarized in Table 2. This certainly provides first-hand information for the designer of a biosensor to develop strategy for selecting either one or multiple candidates to develop test platforms for precise and accurate detection of the COVID-19 disease.

Respiratory Burst. Essentially, polymorphonuclear leukocytes (PMN) circulating in our blood show elevated levels in the blood as a defense response of the host during an episode of infection. PMN inherit different pieces of information in the case of different etiological agents stimulating immunity.[71,72] This causes activation of PMN, and there is an increase in the consumption of molecular oxygen which results in the production of reactive oxygen species (ROS), a process collectively called respiratory burst.[73] Some biosensors in the

https://dx.doi.org/10.1021/acsnano.0c04421
ACS Nano 2020, 14, 7783−7807

Table 2. Potential Blood Biomarkers of SARS-CoV-2

biomarker	reference range	concentration range in COVID-19 patients	ref
C-reactive protein (CRP)	0.068−8.2 mg/L	>15 mg/L	66
procalcitonin	0−0.5 ng/mL	<0.1 ng/mL	66
lymphocyte count	0.8 to 4 × 10^9/L	<0.6 × 109/L	66
interleukin-6 (IL-6)	0−7 pg/mL	>15 pg/mL	66
interleukin-10 (IL-10)	0−9.1 pg/mL	>15 pg/mL	67
cystatin C (CysC)	0.6−1.3 mg/dl	>1.1 mg/dl	68
serum direct bilirubin (DBIL)	5.1−17 μmol/L	>8−60 μmol/L	68
cholinesterase (CHE)	8−18 kU/L	1.5−8 kU/L	68
lactate dehydrogenase (LDH)	140−280 U/L	300−600 U/L	68
creatinine (CREA)	60−120 μmol/L	30−80 μmol/L	68
urea	2.5−7.1 mmol/L	4−22 mmol/L	68
Qualitative changes in the blood profile of COVID-19 patients[a]			
hematologic	WBC count	increases	
	neutrophil count		
	lymphocyte count	decreases	
	platelet count		
	eosinophil count		
	hemoglobin		
biochemical	alanine aminotransferase	increases	
	aspartate aminotransferase		
	total bilirubin		
	blood urea nitrogen		
	creatinine		
	creatine kinase		
	lactate dehydrogenase		
	myoglobin		
	creatine kinase-MB		
	cardiac troponin I		
	albumin	decreases	
coagulation	prothrombin time	increases	
	D-dimer		
inflammatory biomarkers	erythrocyte sedimentation rate	increases	
	CRP		
	serum-ferritin		
	PCT		
	IL-2R		
	IL-6		
	IL-8		
	IL-10		

[a]The information is adapted from the clinical studies of Henry *et al.*[75]

past have been developed for the ROS detection from the virus, and on a similar basis identification of ROS from SARS-CoV2 infection may lead to an alternative route of COVID-19 detection.[74]

BIOSENSORS FOR EARLY DETECTION AND PROGNOSIS OF PANDEMIC VIRAL STRAINS

There are 11 key attributes (Figure 5):

- High selectivity: Selectivity of a biosensor is its ability to exclusively detect the analyte in the presence of other homologous analytes and contaminants. Viruses usually depict similarity in their structures in that they have a nucleic acid genome, a protein capsid which covers the genome, and in some cases lipid covering the genome and a protein coat.[76] This protein coat in the virus distinguishes them from the bacteria.[77] Perhaps specifically identifying and then targeting some proteins on the capsid with other proteins *via* protein−protein interactions may lead to selective detection of the virus.[78−80] A good selectivity is usually achieved by ensuring that the monolayer of probes that target the chosen biomarkers on the analyte is optimally immobilized on the sensor surface.[81,82] Now this is most often a subject of research where it usually takes a dedicated team and a time frame of 6−12 months to develop probes specific to the target. This obviously is a bottleneck for quick development of a biosensor during pandemics such as the COVID-19. However, the advancements in nanotechnology and the pace with which discovery and invention of materials are developing present a good opportunity for researchers to develop chemical probes, specific only for the target under detection.[83,84] While developing specific probes is obviously a challenge, recent work has demonstrated an antifouling coating for the electrodes consisting of biomolecules mixed in a network of conductive nanomaterials.[85] These layers were found to preserve 88% of the original detection signals for IL-6 even after 1 month of exposure to unprocessed human plasma.
- High sensitivity: The spacing, affinity, and specificity of the target probes in the monolayer, often called self-assembled monolayers (SAM) or biorecognition elements, determine the attachment of the analyte on the sensor surface.[86,87] In addition, properties of the transducer (most often electrical or optical) influence the overall sensitivity of the biosensor;[88] for instance, how many molecules should be attached before a response from the sensor could easily be distinguished from its inherent noise.[89] If specificity of the SAM layer

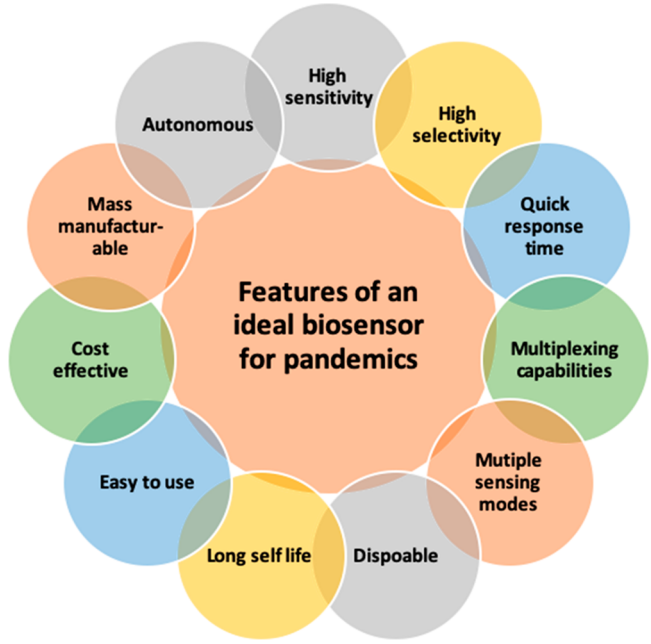

Figure 5. Features of an ideal biosensor required to be developed for effective use in pandemics.

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ACS Nano 2020, 14, 7783−7807

is ensured, then it is possible to detect several small biomarkers (sizes <150 kDa) even at the single molecular level.[90,91] For the detection of biomarkers associated with pandemic strains such as the COVID-19, it is of the utmost importance that the developed sensor can detect the disease-specific biomarkers at a low concentration, preferably at a single molecule level, and produce an output which is easily readable for the measured/required concentration. For instance, one major challenge, if addressed, to detect the viral DNA or RNA directly in whole blood without the need of amplification *via* PCR technology would lead to technologies for rapid detection of the pandemic viral strains. Some complementary tools such as the nanoparticle-based detection tools[92,93] and microfluidics[94,95] might also be well-placed to address some of these fundamental issues involving biological fluid handling on the sensor surface to enhance sensitivity.[96]

- Rapid response time: For a diagnosis tool to be actively used in a pandemic, the response time of the sensor is of paramount importance.[97] Theoretically, most transducers in the sensor respond instantaneously (<1 s) to the applied stimulus, such as upon the interaction of biomolecule with the sensor surface.[98] However, at times, many of these signals require post-processing with advanced electronics and computing systems for accurate analysis of the measured quantity. For instance, temperature correction[99] and background noise identification and removal[100] can often lead to an increase in the sensor response time. Therefore, the design and role of signal conditional circuits are of prime importance to ensure quick response time of the measurement.
- Multiplexing: At early stages of the infection when there is less understanding of the characteristics of the viral strain, it is often the concentrations of common blood biomarkers (such as CRP and interlukien as described for COVID-19) which serve as the signature of the infection. As also described earlier that for COVID-19 detection, a combination of more than two biomarkers is currently used to confirm the disease; therefore, a multiplexed system which allows detection of multiple biomarkers is desirable for a quick, accurate, and early detection of the disease. Multiplexing can be achieved by physically isolating different areas of the sensor surface, where each isolated area acts as a standalone sensor.[101,102] Each of these individual areas could further be made specific to one type of biomarker.[103−105] Measurement can be acquired by either one transducer which will scan the isolated areas of the sensor surface or by incorporation of multiple transducers attached to the individual sensing area.[106,107]
- Multimode sensing for on-chip validation: For the detection of a pandemic strain, it is also necessary that the sensor, while providing a quick response, is also reliable. To increase the reliability in the measurement of the sensor, more than one mode of sensing could provide cross validation of the sensing result.[107] The major trade-off of the multimode sensing is an increase in the size of physical dimensions and computation time of the sensor. This can also lead to a slow response time, increased power consumption, and cost ineffectiveness. However, the good news is that most of these barriers are rapidly being addressed. Electrochemical and optical techniques on single platforms[108] which can easily be translated into multiplexed panels are increasingly getting attention in both academia and industry, which might be able to serve the purpose of increased reliability and robustness required in the healthcare sensors, by instant authentication of the measured data.
- Disposable: The fact that the pandemic viral strains are highly infectious, for example, COVID-19 has a reproductive number higher than that established for SARS and H1N1, that is, between 1.5 and 2, the need for single-use sensors is crucial for avoiding contamination from the sensing systems. The most judicial pathway to develop a disposable sensor system is the modular approach.[109−111] In this approach, electrode and readout modules can be separately designed where electrodes can be made cost-effective and disposable in nature. Candidate materials for developing disposable electrodes could potentially include glass-,[112] paper-,[113,114] plastic-,[115,116] metal-,[117,118] or ceramic-based[119] materials which can be used to immobilize bioprobes specific to the biomolecules of our interest. Among these materials, paper-based biosensing electrodes,[112,113] which have grabbed the attention of the community in recent times, provide the most optimal disposable features, as such electrodes could be classified into burnable trash after use. On the other hand, readout modules in the form of a mobile phone application-based[120] readout will provide many advantages other than cost-effectiveness, such as periodic data collection and connectivity to the centralized healthcare systems.
- Long shelf life and easy to use: The electrodes developed should be easy to use, and their lifetime should be at least 1 month. This will ensure that a large number of testing kits can be prepared and shipped not only to the healthcare facilities but also to the supermarket shelves.[121,122] The ease of use could allow people to self-test and take necessary and informed decisions to self-isolate, which will ensure that the transmission of the disease can be curtailed at its source.
- Cost effective: The advantage of cost-effectiveness of a biosensor is effectively reflected in its affordability.[123] Intuitively, the lower the price of a given biosensor, the more affordable is the device. To keep the price low, so that all members of the community can afford it during pandemics, a biosensing system can essentially be divided into two parts: The first part can be a disposable electrode such as the screen printed electrode or a paper-based electrode, which could potentially be available in the supermarket drug shelves.[124,125] The electrode could have direct contact with a body fluid such as saliva for the quick detection of viral strain. The second part of the sensor system can be an application on a mobile phone which can essentially serve the purpose of a direct readout of signals from the sensor.[126,127] Such applications could potentially be made available by the government or healthcare government body of the affected region or community. On the other hand, a standalone readout and data acquisition system with data loggers for disposable sensors can also be developed, which could be equipped in hospitals or at border control posts of a country.[128] Certainly there are some biosensors that are now easily available in the supermarkets such as the pregnancy test

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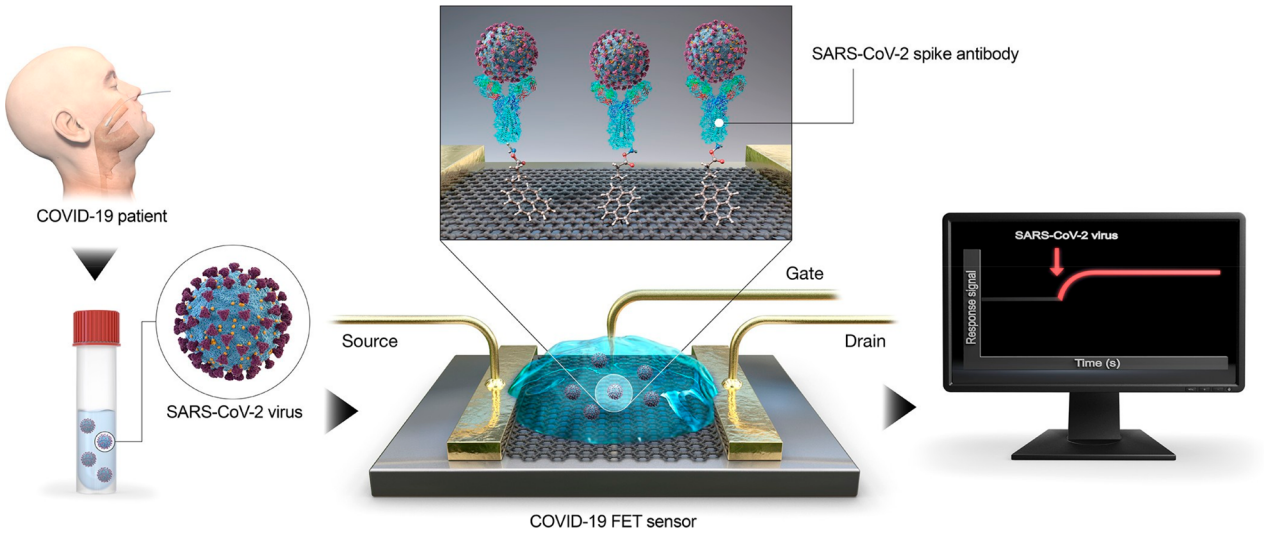

Figure 6. Detection of SARS-CoV2 using FETs: The schematic shows a collection of biological samples from a patient and its application on the graphene-based sensing area of the FET biosensor. Binding events associated with the SAR-CoV2 virus can be captured by the sensor in real time. Reprinted with permission from ref 153. Copyright 2020 American Chemical Society.

strips and lateral flow strips for sexually transmitted disorders and for glucose meters.[125] It is on similar lines that sensors which can rapidly detect viral infection, if developed, could turn out to very useful for self-testing of infectious diseases. In addition, this would decrease the load on the public health bodies to detect the disease in a large-sized population, which could also lead to timely control of the disease spread.

- Mass manufacturing: During pandemics, there is an urgent and large demand for sensors that can detect the fast spreading disease accurately and quickly. Due to lack of availability and reachability of a number of sensors, low rates of testing compared to total population are reported in the case of COVID-19, even in the nations with world leading heath infrastructures.[129,130] In an ideal situation, the number of biosensors is equal to the population number of a given geographical area under test to ensure that all potential members of the community that can spread the disease are identified at an early stage. While there is a significant technological challenge to mass-produce sensors, the latest advancements in the manufacturing such as the 3D printing[131] and machine molding[132] can help in the development of a large number of sensors in a very short duration of time.
- Autonomy and connectivity to central healthcare systems: Autonomy in the biosensing system would ensure a high compatibility between electrodes and the readout modules. Along with autonomy, measurement systems of a sensor in pandemics should be able to connect itself with the central database of a hospital which will collect real-time data from the measurements. For instance, a mobile phone application which collects the data from the sensor can be integrated with a two-way communication channel to (1) send data to the central database and (2) provide prompt therapeutic intervention or facilitate the situation with dispatched paramedical staff.[133−135] Networked healthcare service can also maintain and troubleshoot by additional information about the functionality of a sensor. Furthermore, location, positive disease cases, personal details such as age, gender, and contact information could easily be logged on to the central databases. This would provide the government and health care policy markers with real-time data to quickly and accurately determine necessary actions, such as locking down certain locations to contain and mitigate the fast spread of the disease.[52]

Among the 11 key attributes discussed above, the most important focus of current biosensing innovations should be the sensitivity and specificity of the assays directed toward early detection of COVID-19 disease or future pandemic strains. This is necessary to overcome the uncertainty associated with a wide variety of advanced testing technology including lateral flow assays or direct detection of viral RNA with CRISPR biosensors.[136] Repurposing some of the instrumentation such as the 96-well microtiter plate readers could also potentially provide multiple replicates of a bioassay in a short duration of time. Such absorbance readers are so common that nearly all laboratories which provide routine blood profile testing, including in the remote settings, may utilize this platform for quick testing. For instance, absorbance readers are used in recent work on the quick detection of COVID-19 disease,[137] suggesting that the capital required for new schemes developed already exists within many test facilities. Another important aspect which could potentially lead to a quick solution required in mid-pandemics is the use of soft-biosensing. For instance, artificially intelligent software could be installed in hand-held thermometers which are currently being widely used in the screening of COVID-19 candidate patients. Software could perhaps be developed in such a way that the infrared signals analyzed by the detector provide information on some key skin biomarkers, in addition to the temperature measurement, similar to non-invasive biosensing.[138−140]

Electronic Sensors for COVID-19. Field-effect-transistors (FETs) and three-electrode potentiometric and amperometric systems are the key electronic biosensors widely applicable in the detection of biomolecules and pathogens. The main advantages of an electronic biosensor are miniaturization, low

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cost, and mass manufacturing. Besides, the concepts of a modular sensor for separating electrode and readout on a smartphone can be implemented effectively. FETs for instance are easily fabricated in CMOS foundaries,[141−143] whereas a large number of electrochemical sensors are now commercially available in the market in portable formats.[144,145] In the past a large number of electrochemical sensors have been developed for pandemic viral strains. For instance, Han *et al.* developed a single microfluidic electrochemical sensor for the detection of H1N1, H5N1, and H7N9 combined with zinc oxide nanorods,[146] amplification strategies for sensitive detection of H1N1 with electrochemical sensors by Li *et al.*,[147] electrochemical detection of MERS using carbon electrodes by Layqah *et al.*,[148] detection of SARS by Iskhikawa *et al.*,[149] and many other works which even used disposable screen printed electrodes and paper-based substrates for the detection of viral strains.[150−152] Some of these works have definitely encouraged researchers to develop strategies for the early detection of COVID-19 disease. The work of Seo *et al.* reported a FET biosensor for detecting SARS-CoV2 in clinical samples.[153] This sensor was produced by coating the gate of the transistor made up of graphene sheets, with an antibody that was specific against the SARS-CoV2 spike protein. Desired performance of the sensor was identified with tests conducted with antigen as protein, cultured virus, and nasal swab specimens from COVID-19 patients. The biosensor could detect the SARS-CoV2 spike protein at concentrations of 1 fg/mL in buffer solvents prepared in the laboratory and 100 fg/mL from the biological fluid of the clinical samples. This biosensor was further used for the successful detection of viral strains in culture medium with a limit of detection in clinical samples found around 2.42×10^2 copies/mL. This was indeed a neat example of how biosensors can detect the virus at low concentration without sample pretreatment or labeling, see more details in Figure 6. In another recent work, Mahari *et al.* developed a biosensor using three electrode-electrochemical systems by using disposable screen-printed carbon electrodes.[154] The limit of detection of this biosensor named, eCovSens, was found to be 120 fM in buffer solvents. Even though the detection was performed in nonclinical samples, the electrodes were found stable for up to 4 weeks, suggesting that such electrodes could potentially be mass-produced, shipped, and then distributed in the community within a reasonable time frame during the mid-pandemics.

Optical Biosensors for COVID-19. A large number of optical biosensors primarily based on the principle of plasmonics,[155] essentially where the transduction principles use optical components such as waveguides,[156] fiber optics,[157] photonic crystals,[158] and lasers[159] are classified into optical sensors. Optical biosensors such as the surface plasmon sensors (SPR), including the localized surface plasmon resonance (LSPR), are commercially available since early 1990s,[160] and they have been widely used to detect viral strains such as those associated with H1N1,[161] SARS,[162] MERS,[163] and influenza[164] in laboratory settings.

While many of the developed plasmonic techniques with advanced surface chemistry provide high sensitivity, selectivity, and quick response time for the detection of the viral strains, their use in point-of-care applications remains challenging. This is due to the large size and cost of the instrumentation which is involved in the development of plasmonic systems. Even though the use of these sensors remains elusive for the mass production and self-testing by community members, these sensors are now well-developed for widespread use in laboratory-based settings for accurate detection of viral strains during pandemics. Recently, Qiu *et al.* have developed a biosensor for accurate diagnosis of COVID-19 disease where plasmonic photothermal (PPT) effect and LSPR are combined as transduction principles in the sensing scheme.[165] Essentially, the DNA receptors are used to detect selected sequences from the SARS-CoV2 through nucleic acid hybridization. With the mandatory use of nanoparticles and light in LSPR sensing, there is a generation of well-known thermoplasmonic at the plasmonic resonance frequency. The authors claim to elevate the *in situ* hybridization temperature using this thermoplasmonic heat which further assists in facilitating accurate discrimination of two similar gene sequences. This LSPR biosensor showed a good limit of detection, down to the concentration of 0.22 pM, exhibiting a high sensitivity toward the selected SARS-CoV-2 sequences in a multigene mixture; see more details in Figure 7.

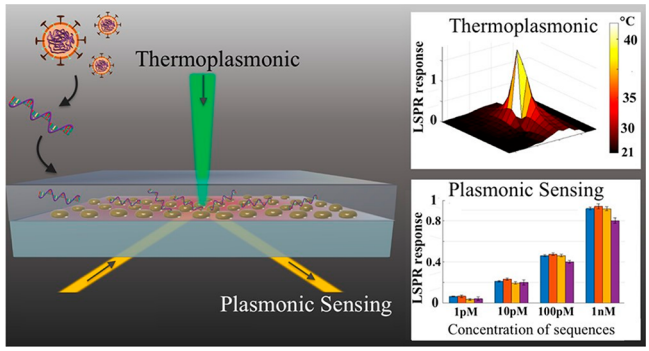

Figure 7. LSPR detection of nucleic acid sequences from SARS-CoV2 virus. The schematic shows the architecture of LSPR substrate consisting of gold nanoparticles where light is illuminated on the substrate for generation of local heat and detection of binding nucleic acid binding events. The graph also shows the LSPR response to the theroplasmonic effect and toward detection of nucleic acid sequences at low concentrations. Reprinted with permission from ref 165. Copyright 2020 American Chemical Society.

It should be noted that with current advancements in the nanofabrication[166] and combination of LSPR with microfluidics,[167] further enhancement of sensitivity and potential integration in portable formats is feasible.[168,169] Therefore, LSPR holds a great potential to improve the diagnostic accuracy in the clinical tests and relieve the pressure on PCR-based tests that usually need a well-equipped laboratory and well-trained personnel, which usually remains challenging due to a shortage of resources during pandemics.

In addition to the direct sensing of analytes which confirm viral infection, optical sensors can also play a significant role in high-resolution imaging of the viruses during pandemics. For example, Wei *et al.* demonstrated how optical sensors can be used for virus imaging based on the laser diode excitation.[170] The developed system is in the form of an optomechanical attachment which can easily be integrated to the existing camera module of a smart phone, see more details in Figure 8. The special resolution of the imaging platform is <50 nm, allowing easy detection of individual viruses using a smartphone.

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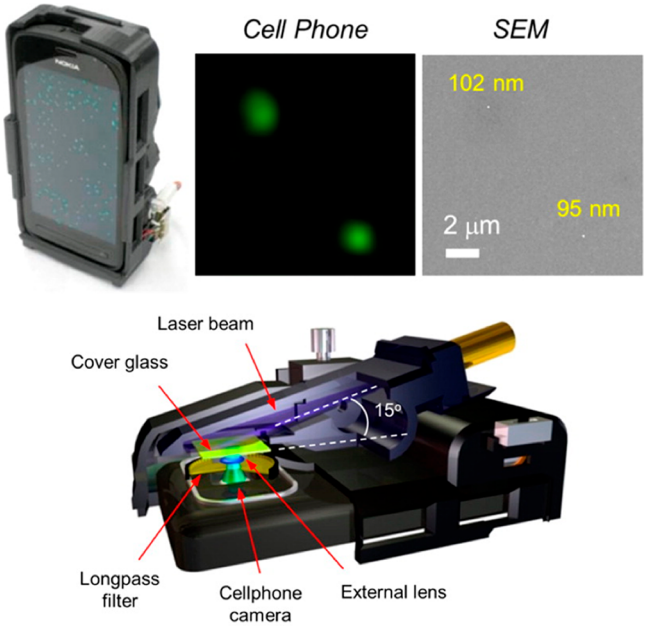

Figure 8. Imaging single viruses using a smartphone. A smartphone-based optomechanical attachment with a resolution of <50 nm for the detection of individual viruses. Reprinted with permission from ref 170. Copyright 2013 American Chemical Society.

BIOSENSORS FOR DRUG ANALYSIS AND ACCELERATING DRUG DISCOVERY FOR VACCINES

As biosensors provide detailed information on the binding affinity of the biomolecules to a given substrate and their associated kinetics, their use in drug discovery applications is ubiquitous. In addition, as the design of a given transducer can be tuned according to its intended application, different types of biosensors can be implemented in various levels of the drug discovery process to enhance the discovery of a potential vaccine.[171] In addition, as viruses evolve,[172,173] the need for a new drug is also inevitable, and it is in this context where sensing technologies can provide a solid backbone to bear the burden of a timely discovery of new drugs to contain an outbreak of a disease. In the drug discovery process, the research and development phase of the drug discovery process normally takes 3−5 years, preclinical studies take 1−2 years, and clinical trials take 2−3 years, followed by review and approvals which take over 6 months to 1 year.[174] Time of these stages can significantly be reduced with the use of advanced bio/chemical sensing technologies. We provide key attributes of biosensors specific to the drug discovery stages that can possibly assist in speeding up the drug discovery process:

- Ligand fishing: Identification of potential drugs from natural resources such as the plant extracts is a critical, challenging, and time-consuming task in the process of drug discovery.[175] For example, discovery of a bioactive constituent from traditional Indian and Chinese medicines has contributed to their well-known therapeutic effects in viral diseases, including for SARS CoV2-virus.[176−178] Identification of such bioactive compounds has largely been dependent on techniques such as ultrafiltration,[179] liquid chromatography (LC),[180] and mass spectrometry (MS),[181] known as ligand fishing. It is in this context that all biosensors represent themselves as a useful tool for the discovery of bioactive compounds from a complex mixture of natural products while providing enhanced efficiency and reduced sample preparation time at a low cost as compared to the existing techniques such as the LS and MS. While biosensors combined with the nanomaterials have also shown high selectivity toward the targets, sensors for screening active compounds from natural products have not been reported so far. However, by combining biosensors with microfluidics, it is possible to develop such tools due to common procedures such as incubation,[182] washing,[183] mixing,[184] and compartmentalization[185] of the solution being easily achieved by microfluidics systems.
- High-throughput screening of candidate drug compounds: In contrast to the extraction of compounds from the natural products, in laboratory synthesis of chemical compounds for possible use as drugs, the ability of the compound to affect the identified target using biosensors can be tested. Here high-throughput array-based sensing technologies such as the FETs[186−188] surface plasmon sensors[189,190] have an advantage over the conventional techniques of LS and MS where effectively more than 10,000 compounds can be screened in 1 week as compared to 3−4 years of testing. In addition to compound screening, cross-reactive activity of the candidate compounds,[191] to check their interfere with other homologous targets, could also be tested using such sensing platforms. An example of a high-throughput biosensor chip consisting 9000 electrodes is shown in Figure 9.
- Affinity and kinetics: Affinity and kinetics information on a compound is vital in understanding its use as a potential drug.[192] For instance, biosensors can closely be made to perform experiments based on the bioinfor-

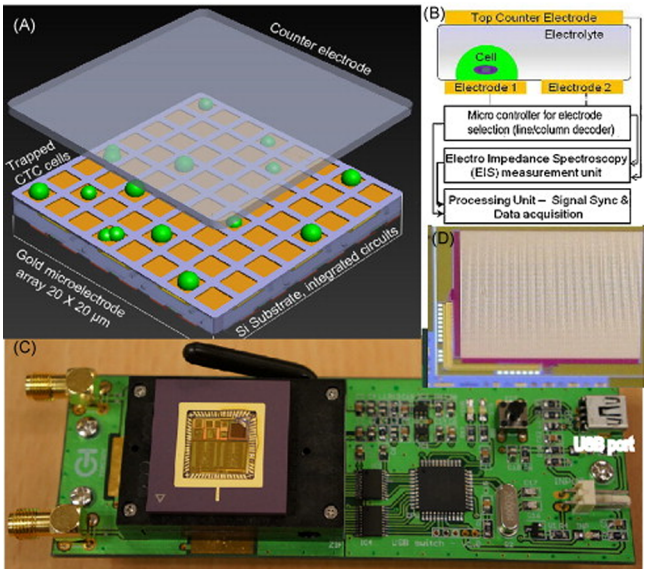

Figure 9. High-throughput biosensor: This example shows (A) microelectrode array used for cell detection; (B) sensor layout and the addressing scheme employed in the CMOS sensor chip; (C) complete CMOS packaged biochip; and D) microphotograph showing 9000 electrodes in the chip. Reprinted with permission from ref 188. Copyright 2012 Elsevier.

https://dx.doi.org/10.1021/acsnano.0c04421
ACS Nano 2020, 14, 7783−7807

matics modeling algorithms.[193] In preclinical trials, sensing platforms can assist in evaluating the binding affinity and kinetics of the antibodies or to assess the developed vaccine to the target protein or loci on the virus. Statistically relevant information on affinity and kinetics can also be generated in a short duration of time with use of multiplexed biosensing arrays.[194]

- Monitoring chemical parameters in the pharmaceutical production process: The production of drugs operates in a stringent safety, sterility, and precision-oriented environment. Therefore, use of biosensors in monitoring chemical parameters such as pH, temperature, and enzymatic activity in real time during the process of drug production is significant to improve the quality of the drugs.[195] Other bio/chemical sensors which measure gases such as oxygen and concentration of byproducts in the process are also useful in the identification of physical and chemical errors in the production line of the drugs.[196] A significant challenge and opportunity for some of the advanced biosensors is to essentially integrate themselves in the production line in the form of a sensor network. Further, with integration of machine learning programs, data from these biosensors could potentially be used to improve the delivery time of drugs in large quantities required during pandemics.[197]
- Bedside drug monitoring in hospitals: Another significant opportunity for biosensors in pandemics is in drug analysis by biomedical systems equipped along the bedside in the hospitals.[198,199] An appropriate quantity of drug injected in the body of the patient can significantly improve the recovery time of the patient. Most often, it is essential to monitor various parameters of the body such as the blood pressure, heart rate, temperature, oxygen concentration, and other small molecules in the blood profile.[200] Establishing a real-time relationship between concentrations of aforementioned parameters is essential, and this is where biosensors, if combined with the internet of technology, can effectively be used for the measurement and analysis of drugs in real time.

MICROFLUIDIC BIOSENSORS FOR PANDEMICS

Microfluidics is a technique for precise control and manipulation of microscale fluids.[201] The basic operation units such as preparation, extraction, reaction, and detection in various analysis processes are integrated on a microchip. Through the micromachining process, the micro- to submillimeter-level fluid channels, pumps, valves, sensors, detectors, and other units can be fabricated on the substrate of silicon, metal, polymer, or other materials. So far, microfluidic platforms have been applied using various analytical techniques including electrochemical analysis, fluorescence analysis, MS, and chemiluminescence.[202] Microfluidic platforms can be classified into capillary, pressure-driven, centrifugal, electrokinetic, and acoustic systems on the basis of their liquid propulsion principles.[203] In the past decades, microfluidic biosensors have been developed for the detection of infectious diseases in the medical field. Infectious diseases are usually identified by bacteria (*e.g.*, *Streptococcus agalactiae*, *Escherichia coli*, *Haemophilus influenza*), food borne pathogens (*e.g.*, salmonella, listeria, cholera toxin), and viruses (*e.g.*, hepatitis C, influenza, dengue virus).[204] For example, shear horizontal surface acoustic wave biosensors were established for multiplex detection of anti-p24 and anti-gp41 antibodies of HIV.[205] The designed dual-channel biochip consists of a sensing area sensitive to biological binding events, along with *in situ* reference control coating and miniaturization configuration. The microfluidic biosensor can detect HIV biomarkers within 5 min using only 6 μL of plasma sample, which raises the prospects of the next generation of economical, rapid, and point-of-care diagnosis to prevent HIV pandemics. A silicon nanowire-based microfluidic biosensor was also developed to detect the RT-PCR product of dengue serotype 2.[206] A detection limit as low as 10 fM was reported within 30 min, which was 3 orders of magnitude lower than that obtained by using ^{32}P-labeled probes and 4 orders of magnitude lower than that achieved by ethidium bromide staining. During the COVID-19 pandemic, rapid diagnosis is of great significance in early diagnosis of SARS-CoV2 for timely and appropriate treatment. Although culture-based methods and molecular techniques are commonly used in clinical diagnosis, recently introduced microfluidic biosensors have the advantages of cheapness, portability, rapid response, high precision, high reproducibility, low reagent/sample consumption, easy application, and high-throughput parallel processing.[207] Therefore, microfluidic biosensors can meet the World Health Organization guidelines "ASSURED" (*e.g.*, affordable, specific, sensitive, user-friendly, rapid and robust, equipment free, and deliverable to end user),[208] which have tremendous potential to be implemented for SARS-CoV2 detection in point-of-care COVID-19 diagnosis. However, further efforts should be devoted to improving sensitivity and specificity, strengthening stability and efficiency, and shortening fabrication time and analysis time for monitoring pandemics. It is also necessary to reduce the matrix effects of actual samples. Moreover, it remains challenging to develop fully automated microfluidic biosensors for on-site monitoring of pandemics.

WASTEWATER BIOSENSORS FOR EARLY WARNING OF OUTBREAK

Wastewater-based epidemiology (WBE) has been implemented for the determination of various biomarkers, including illicit and licit drugs, pharmaceuticals, and personal care products, markers of population size, industrial chemicals, and biologicals.[209,210] Human viruses (*e.g.*, astroviruses, enteroviruses, noroviruses, rotaviruses, salivirus) have also been detected in wastewater, indicating that WBE holds great potential in the early determination of viral outbreaks by routinely monitoring the concentration and diversity of viruses in wastewater.[211] Generally, WBE can provide information about viruses on a population scale and assess the temporal and spatial trends of virus occurrence in the watershed within the catchment of sewage treatment plants. The outbreak of COVID-19 caused by SARS-CoV2 has brought about a significant risk to human health. It has been suggested that SARS-CoV2 can be detected in fecal samples of confirmed COVID-19 cases from many countries including China,[211,212] United States,[213] Germany,[214] Singapore,[215] and Korea.[216] The research involving 10 pediatric COVID-19 confirmed cases provided the potential evidence for fecal viral shedding of SARS-CoV2.[217] Even if the results of the nasopharyngeal testing became negative, the rectal testing of eight children was still positive, indicating that viral shedding of gastrointestinal tract and fecal−oral transmission might occur. The presence of

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SARS-CoV2 in fecal samples may increase the virus load on the wastewater systems of infected cities.

Recent evidence suggests that determination of SARS-CoV2 in wastewater is helpful to study the virus transmission in humans since SARS-CoV2 can be excreted in feces or urine. Several attempts have been made to detect SARS-CoV2 in wastewater including the Netherlands,[218] United States,[219] France,[220] Australia,[221] Spain,[222] and Sweden. The report of SARS- CoV2 detection in sewage was published by Gertjan Medema *et al.*[218] in the Netherlands. Specifically, the targets of gene N (N1−N3) and gene E were tested by RT-qPCR in the WWTP sewage samples to determine the presence of SARS-CoV2. Neither gene N nor gene E was detected on February 6, while gene N1 was detected in six sites, and genes N3 and E were detected in five and four sites on March 15 and 16, respectively. This is consistent with the first case in the Netherlands reported on February 27. In the United States, SARS-CoV2 at high titers was determined by RT-PCR in wastewater samples from a major urban treatment facility in Massachusetts.[217,218] Their work demonstrated that longitudinal analysis of wastewater can provide an estimate of the population level of SARS-CoV2 burden without available on-site testing. The appearance of SARS-CoV2 RNA was verified in six WWTP wastewater samples from a low prevalence area Murcia in Spain.[222] Compared to the COVID-19 cases announced by the municipality, the environmental surveillance results clarified that SARS-CoV2 had spread among the population before the initial cases were reported in many cities. Therefore, WBE could be used as an early warning tool for the presence and prevalence of COVID-19 infections and provide a more accurate estimate of the spread extent of COVID-19 infections than clinical testing.[223] RT-PCR assays have been routinely employed for SARS-CoV2 detection in numerous research and disease control centers since the outbreak of COVID-19,[223] as also discussed in our aforementioned sections. However, RT-PCR has some disadvantages which may limit its applications globally. For example, complete facilities, expensive equipment, skilled technicians, and laborious procedures are essential for RT-PCR. With the rapid growth of COVID-19 confirmed cases worldwide, this method has been unable to meet the requirements of detecting numerous suspicious cases in a short time even though in some cases, PCR has demonstrated high sensitivity and specificity. Essentially, the requirements for complicated sample handling in the laboratory, skilled personnel, and a long period of data processing and analysis (4−6 h) are not conducive to real-time and effective monitoring of samples on location. Therefore, it is critical to develop efficient transportable and robust analytical tools to accurately and quickly trace low-level SARS-CoV2 sources through WBE to confirm these suspected cases and screen asymptomatic infected cases without centralized laboratories. Using a WBE strategy in creating an early warning and intervention system will require a rapid biosensing method for the on-site detection of viruses at the wastewater collection outlets.[209,212] We have recently proposed the use of a paper-based device which can be used for WBE, as developed in our previous work,[224] see Figure 10 for more details. Recent advances on signal amplification strategies have also lead to enhanced sensitivity in paper-based analytical devices for various sensing applications.[225] Due to the advantages of low cost, rapidness, sensitivity, specificity, accuracy, and simplicity, biosensors have been applied in detecting different analytes for clinical diagnosis, food safety,

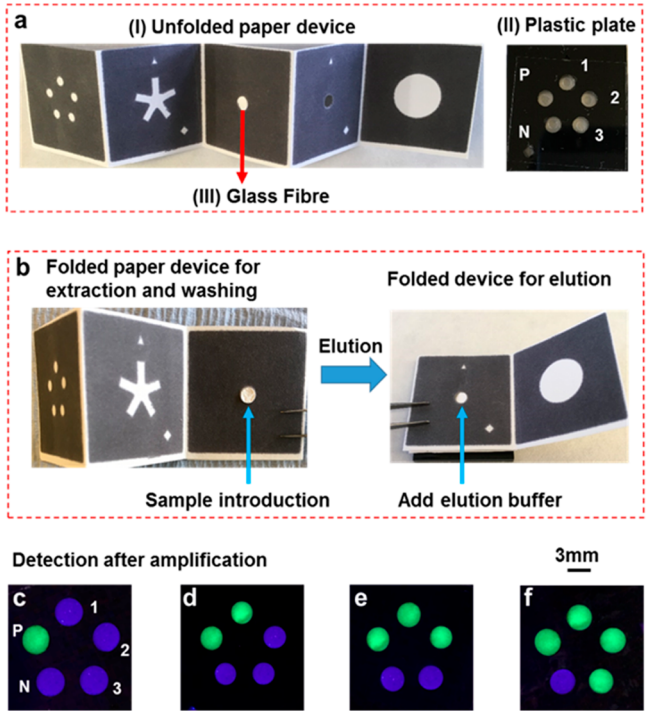

Figure 10. Design of an integrated sensor for the detection of multiplex infectious disease pathogens. (a) Components of the sensor and (b) illustration of the complete sample processing from sample introduction to pathogen detection. Reprinted with permission from ref 224. Copyright 2018 American Chemical Society.

and environmental monitoring. Recently, biosensors have been employed for electrochemical, optical, electrical, thermal, acoustic, and piezoelectric determination of infectious diseases such as AIDS, dengue, Ebola, influenza, and malaria. The biomarkers of these infectious diseases are usually identified in blood, feces, plasma, serum, sputum, saliva, urine and nasal mucosa. When detecting wastewater samples, the matrix effects of the wastewater can also be minimized using an ultrahigh-affinity probe for specific targets.[226] Moreover, biosensors can be extended for the multiplex detection of infectious diseases. For instance, Men *et al.*[227] presented a multifunctional fluorescent protein nanowire (FNw) for multiplex detection of hemagglutinin 1 protein from the influenza virus along with p24 and gp120 protein from HIV. The FNw integrated with numerous green fluorescent protein molecules could amplify signal and enhance sensitivity. In addition, biosensors can be developed into integrated medical devices for point-of-care detection of infectious diseases in resource-limited regions. For example, Choi *et al.*[228] presented an integrated paper-based sample-to-answer biosensor for nucleic acid testing. The fast technology analysis (FTA) card and glass fiber were integrated into a lateral flow strip for nucleic acid extraction, amplification, eye detection, and smartphone quantification. A hand-held battery-powered heating device was also used for LAMP amplification in POC settings. Hence, biosensors can be employed as early warning sensing systems to help the government take effective measures in advance to prevent the spread of infectious diseases.

Biosensors hold potentials for *in situ* quantitative analysis of SARS-CoV2 in wastewater. Although wastewater is a complex matrix, we have recently proposed a paper-based device which

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has shown great potential to detect pathogens in wastewater. Essentially, we developed a fast "sample-to-answer" analysis method which can provide quantitative monitoring of nucleic acids and genetic information through the analysis of sewage waste.[229] Our results were further cross validated with a robust electrophoresis and agarose gel image assay, showing promising reliability for wastewater analysis. The proposed biosensors will show advantages including affordability, rapid analysis time, excellent sensitivity, superior specificity, and low reagent/sample consumption. These benefits highlight the biosensors' extraordinary capability of economical, portable, and user-friendly detection of SARS-CoV2 in wastewater compared to traditional detection methods. Biosensors will help identify COVID-19 infected patients on the spot to provide timely medical advice and monitor the spread of SARS-CoV2 at early stage. Therefore, sewage sensors for SARS-CoV2 detection at the population level have a clear potential for early warning of COVID-19 pandemic. For example, a recent study demonstrated that live SARS-CoV2 was isolated from the feces and urine of infected people, which would then enter the wastewater treatment system.[230] Although SARS-COV-2 is usually present in sewage in the form of RNA particles, there currently is no evidence which shows live SARS-CoV-2 present in sewage. Within this viewpoint, further research on the environment for the analysis of disease biomarkers is required to effectively use WBE biosensors for an informed status of the disease within a community.

NANOSCALE VISUALIZATION AND CHARACTERIZATION TOOLS

There are three principal techniques commonly used individually or in combination for the direct visualization, diagnosis, and characterization of virus structure: XRD analysis, EM, and AFM. The exceptional capabilities of these instruments allow not only in-depth analysis of virus structure and function but also investigation of viral impact on host cells and extracellular environment, useful in drug discovery applications.[28,231−241]

Electron Microscopy. An EM is a tool that uses a beam of accelerated electrons as a source of illumination. Due to the short wavelength of electrons in comparison with visible light photons (up to 100,000 times), the resolving power of EM is higher than optical microscopes and can visualize the structure of particles at nanometer scales. The capability of EM to reveal the structures of nanoparticles makes it a powerful instrument for detection, diagnosis, and analysis of viruses (as viruses are of the same scale as that of many inorganic nanoparticles such as gold, extensively used in research). In comparison, with the molecular and serological approaches used for virus diagnosis which require specific probe to recognize virus, EM methods do not need organism-specific reagents for recognizing the pathogenic agent. It means for the case of an unknown disease, molecular tests need information about the potential agents to determine the appropriate tests, while EM provides an open view of whatever might be present in a given sample under investigation.[237] Besides the capability of EM in direct detection of virus by visualization, it provides information about the ultrastructure of the virus, its structural dynamics related to the attachment, and replication processes. This makes EM a useful tool in the discovery and design of antiviral agents and vaccines.[236−238,242,243] The main techniques of EM microscopy to analyze the virus structures are negative staining, thin sectioning, immune electron microscopy (IEM), cryoelectron microscopy (cryo-EM), tomography, and scanning electron microscopy (SEM). In negative staining, the background is stained, leaving the actual specimen untouched and thus visible. Thin sectioning is used to reduce the viruses into thin layers to be transparent for electrons. The principle of IEM is the formation of immune complexes of the virus with its associated antibody. The cryo-EM is an imaging procedure used to generate high-resolution 3D images of samples, commonly biological materials and cells. In this method, a series of two-dimensional (2D) images of sample are captured while it is tilted at different angles, and then, the 2D images are merged to reconstruct 3D image.[243−245] On the other hand, SEM has also been used for virus quantification procedures.[246] It should be noted that SEM if combined with the abilities of transmission electron microscopy (TEM) can also provide high-resolution images of virus structure with minute structural details for improved characterization of viruses.[237] In the past using EM techniques, SARS was classified as a coronavirus.[247,248] EM was used in the recognition of MERS,[249] and the image of COVID-19 virus was taken by TEM,[250,251] see Figure 11 for more details.

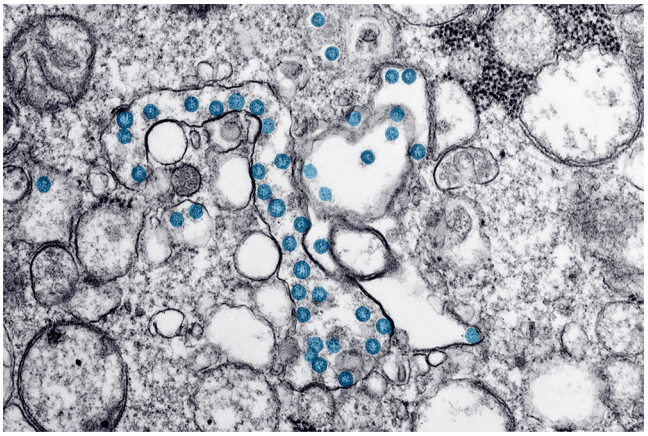

Figure 11. Visualization of SARS-CoV2 with TEM. The virus is shown in blue color. Reprinted with permission from ref 38. Copyright 2020 American Chemical Society.

Recently cryo-EM was used to describe multiple monoclonal antibodies targeting SARS-CoV2, specific to the S protein identified from memory B cells, from an individual who was infected with SARS-CoV in 2003.[42] One antibody, named S309, potently neutralizes SARS-CoV2 and SARS-CoV. Using cryo-EM and binding assays, it was also revealed that S309 recognizes a glycan-containing epitope which is conserved within the sarbecovirus subgenus, without competing with the receptor attachment.[250] In ref 252, the cryo-EM is used to determine a 2.9 Å-resolution structure of the RNA-dependent RNA polymerase nsp12 of CoV 19, which catalyzes the synthesis of viral RNA, in complex with two cofactors, nsp7 and nsp8. In ref 253, the 3D structure of the causative agent in SARS infection and the comprehensive information about the morphological surface of SARS-CoV was obtained. This included enhanced visualization of the trimeric structure in the 10−20 nm spikes of the virus surface. These results support the characterization of the SARS agent and development of antiviral strategies,[253] and we envisage that such techniques can easily be extended to reveal the details of CoV 19 viruses.

https://dx.doi.org/10.1021/acsnano.0c04421
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X-ray Crystallography. The XRD from single crystals is a promising method to obtain the highest resolution of the virus structure and visualize the macromolecular assemblies at an atomic level. Generally, there are five steps to identify the virus structure using X-ray crystallography techniques which include: preparation and purification of virus particles, crystallization and mounting the particle crystal, measurement of diffracted data, calculation of phase through molecular replacement (MR) or isomorphous replacement,[254,255] and finally, map interpretation and building the model.[256−258]

To prepare and purify the viruses from the extracellular/tissue culture media, purification kits are readily accessible from the commercial sources.[259] During the preparation steps, to maintain the icosahedral symmetry of the virus, the handling procedures should be performed as gentle as possible. Following the purification stage, crystallization is required to bring the aqueous protein solution to supersaturation. There are two phases in crystallization: nucleation and growth. In nucleation, the crystal nucleus is formed at the critical size of molecular aggregation, then a subsequent process of growth is initiated. For growing the crystals of virus samples, there are four methods, batch crystallization, dialysis, liquid−liquid diffusion, and vapor diffusion.[260]

To improve the resolution and prevent the secondary radiation damage to the virus sample in the collection process of X-ray data, the crystallographic data are collected at cryogenic temperature, typically around 100 K. Growing high-quality virus crystal is one of the challenges in X-ray crystallography of the virus particles. Methods currently in use are time-consuming and based on trial and error procedures. Nevertheless, cryo-EM/ET can be a powerful complementary method for virus protein crystallography.[261] Information provided by 3D cryo-EM can be integrated with the collected X-ray data to improve the built model of virus particles.[262]

Serial femtosecond crystallography (SFX) is another type of X-ray crystallography that uses X-ray free-electron lasers (XFELs). Due to the pulse duration in femtosecond and unparalleled brilliance of XFEL beams, this technique is useful to study the viruses as both a crystal and single particle. Using time-resolved SFX, the changes during life cycles of viruses such as their response to the variation of pH and viral protein interaction with the receptors can be detected.[234,263−265] Therefore, the combination of high-resolution static structure of viruses obtained by cryo-EM and dynamic results of conformational changes at room temperature using SFX can be a breakthrough toward producing time-resolved virus structures.[266] The use of X-ray crystallography methods to study the family of coronaviruses shows the ability of the tool for in-depth analysis of COVID-19.[267−269] Recently, in ref 270, the 3D crystal structure of the SARS-CoV2 unliganded Mpro at 1.75 Å resolution is determined (see Figure 12 for more details), and it was used to guide the optimization of a series of α-ketoamide inhibitors. Such structural analysis forms a strong basis for further development of these compounds to antiviral drugs. However, both common EM and XRD methods are based on the averaging of the numerous particles present in the electron micrographs or crystal. Therefore, there is a limitation in the information obtained from these structural differences that can exist between the individual particles in a large population. Besides, these methods require environments which are far away from the physiological conditions in which the virus normally operates and precludes the characterization of their dynamic properties in real time.

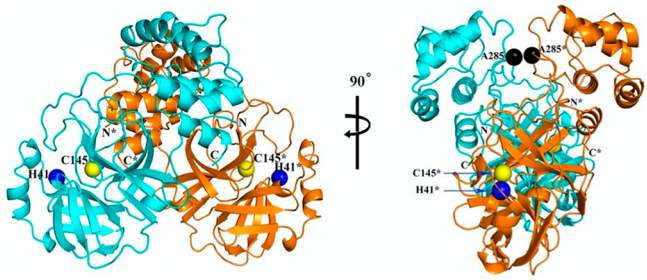

Figure 12. The 3D structure of SARS-CoV2 Mpro, in two different views. One protomer of the dimeriz shown in light blue and the other one in orange. Amino acid residues of the catalytic site are indicated as yellow and blue spheres, for Cys145 and His41, respectively. Black spheres indicate the positions of Ala285 of each of the two domains III (see text). Chain termini are labeled N and C for molecule A (lightblue) and N* and C* for molecule B (orange). Reproduced with permission under a Creative Commons CC-BY license from ref 270. Copyright 2020 American Association for the Advancement of Science.

This complicates structural studies of viruses that lack a well-defined symmetry such as the SARS-CoV2 as an enveloped virus. A third, direct imaging technology that provides significant impact on virus study is AFM. AFM provides possibilities for studying the COVID-19 virus particles, complementing classical EM and XRD studies.

Atomic Force Microscopy. AFM has become a powerful instrument for the visualization and characterization of nanoscale imaging of samples in air and in liquid.[271] Typically, in AFM measurement, the deflection of microcantilever is used to probe the interaction between the surface of the sample and the nanometric tip located at the end of microcantilever. Based on the time scales, type, and range of interaction, an extensive range of information including atomistic details of the surface, mechanical, physical, chemical, thermal, and viscoelastic properties of nano/bio materials can be obtained.[272−287] The earliest invented mode of AFM was the contact mode, where the tip is raster scanned through the sample surface while maintaining a constant force applied to the sample through deflection detection by adjusting the height of the tip. The main challenges in using contact mode to image soft materials such as viruses and other biological specimens is the applied force to avoid the reversible or irreversible deformation, unwanted friction, and damage to the sample.[288] To improve the image resolution and minimize the applied force and friction, the dynamic AFM was invented.[289] Generally, in dynamic AFM, the microcantilever is oscillated usually close to its resonance frequency while scanning the sample surface.

Two major dynamic AFM modes are amplitude modulation atomic force microscopy (AM- AFM) (also known as tapping mode) and frequency modulation atomic force microscopy (FM-AFM).[289] In the dynamic mode, due to the decrease of the contact time between tip and sample to the small fraction of oscillation period, the friction is minimized and the risk of damage to the sample is significantly reduced. In addition, of these two common modes of dynamic AFM, recently, several advanced techniques are developed to increase the resolution and explore more information from the materials during imaging. Due to the dynamic nature of these advanced techniques, they can be classified as multifrequency methods,[281,289] where the cantilever is excited at different frequencies and different signals are used as feedback

https://dx.doi.org/10.1021/acsnano.0c04421
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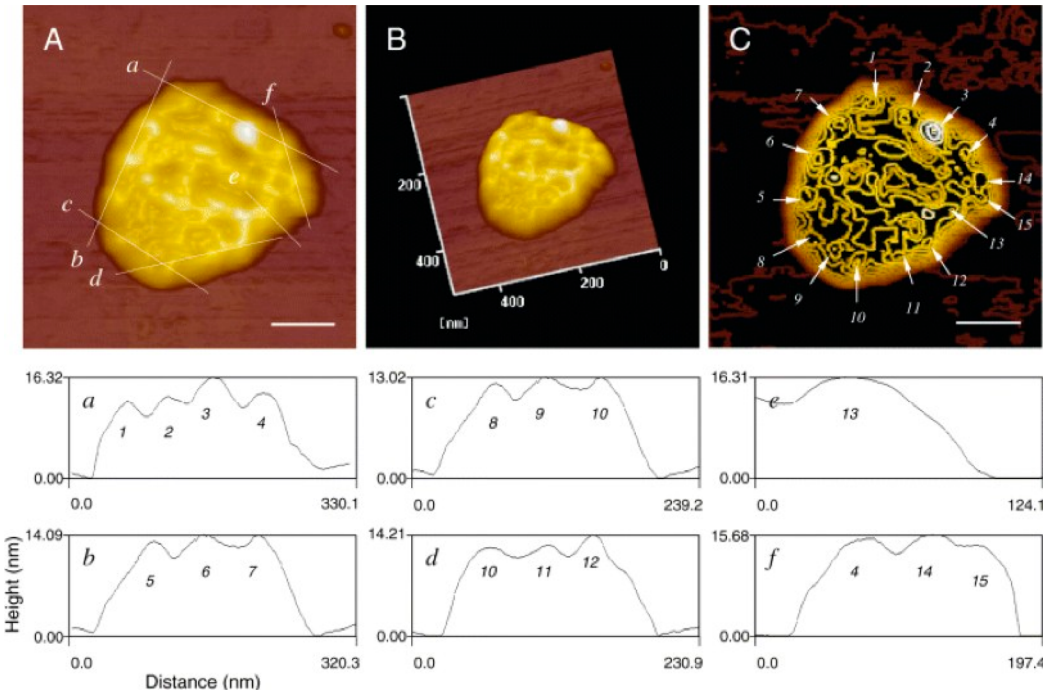

Figure 13. (A) 2D and (B) 3D AFM images and contour map (C) of a single SARS-CoV virion. Scale bar = 100 nm in (A) and (C). The corresponding cursor profiles (middle and bottom row) provide quantitative measurements of the dimensions for the spike proteins (1−15) displayed in (C). Reprinted with permission from ref 302. Copyright 2020 John Wiley and Sons.

parameters.[25] In addition to the ability to visualize and image virus particles, AFM has the capability to measure and quantify the mechanical and structural properties of virus particles.[290,291] Moreover, AFM can be used to manipulate and dissect biological materials including viruses.[292−295]

To extract and quantify viscoelastic, chemical, and physical properties of viruses, the force−distance curve-based AFM (FD-based AFM) and recently multifrequency approaches are the common methods. In modern FD curve-based AFM, numerous FD curves are recorded during imaging the biological sample.[296] Based on the principle of FD-based imaging, the method to map chemical and biological properties is developed.[297,298] In this method, the tips are functionalized by specific ligands. Then, based on the adhesion and mechanical strength of bonds formed between functionalized tip and receptors of the samples, the biological properties can be explored during imaging.[25,291] The main challenge of FD-based AFM is the high volume and time of data acquisition.[25] To increase the speed of imaging and reduce the amount of collected data during measurement, high-speed AFM and multifrequency methods are invented.[299,300] Several multifrequency AFM methods have been proposed, however due to their complex physical principle, theoretical development and mechanism of interpretation of data are under more investigation.[299,301] The previous studies of coronaviruses using AFM techniques[302−305] show the significant potential of AFM to image, visualize, and explore the morphological features of the SARS-CoV virus; see Figure 13 as a typical AFM characterization. Generally, visualization/characterization devices are useful as they provide following information: (1) quantify the surface and subsurface properties of the affected cell by COVID-19 in real time; (2) detect multiple dynamic interactions between SARS-CoV2 virus and host cell; and (3) study the mechanism of SARS-CoV2 virus to cross the cellular membrane and deliver its genome inside the host cells and (4) its replication inside cell and characterization of the nucleic acids of SARS-CoV2 virus, to understand how it is condensed and packaged inside capsids. Recent proposed techniques to nanoscale map directional flow patterns also can be used to study the effects of different pH and ion-specific sieving properties of the host cell on the binding of S protein of SARS-CoV2 virus with receptor at the surface of the cell.[306] In fact, very recently Yang *et al.* characterized molecular binding and inhibition interactions of SARS-CoV2 with ACE2 receptors with AFM.[307]

TECHNOLOGY AND POLICIES

While academia has made tremendous progress in the field of biosensors, a major challenge remains in overcoming the difficulties associated with translating the laboratory research quickly into commercially viable prototypes by industry and addressing the complex regulatory issues required for clinical settings, especially during pandemics. The good news is that slowly the technology transfer investments from government and industry is on the rise. In addition, regulatory policies from the government in many countries are becoming increasingly business friendly with the academics. This is evident from the fact that a lot of organizations are now identifying and bridging gaps between the academic research and industrial needs *via* workshops and university-funded proof-of-concept projects. Moreover, specialized urgent funds without limits are set up by many governments during an infectious disease outbreak such as for the COVID-19. On the other hand, instrumentation such as SPR, AFM, XRD, and EM, while well-established and developed from the infrastructure viewpoint, has a high cost which limits its use mostly to laboratory testing.[308,309] Still, large-scale industries, especially the ones involved in the vaccine development and testing, can potentially reveal morphological features.[310]

https://dx.doi.org/10.1021/acsnano.0c04421
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However, one significant challenge in the technology transfer of most of the biosensors and advanced instrumentation is the capacity building of human resources. The main reason is that practical hands on skills from subjects which are fundamentally far from each other such as biology and electronics are often required with the operator of the sensing instrumentation. This limitation is also being addressed by many educational institutions where courses and projects are encouraged to be interdisciplinary in nature. Other technical challenges for fast technology transfer during pandemics which remain elusive are quick adoption of the biosensor manufacturing protocols and reliability of the sensors. As most of the quickly developed technologies are customized for the pandemic strains, reproducibility, stability, and reliability tests can take more than a few months of time to generate statistical data for making informed decisions. It is here that fundamental science and engineering need to further discover materials which are inherently physically and chemically stable and invent technologies to provide more reliability in the measurements.

In addition, modular sensing systems also provide a flexibility in usage for different analytical biomarkers. For instance, in a single measuring module, electrodes could potentially be used for the detection of multiple biomarkers of a pandemic strain by simply changing the biorecognition layer or the surface chemistry on the electrode. On the other hand, translation of nanoscale analytical technologies to clinical settings depends on the ability to simplify complex analytical devices for non-experts to use and control. Automation and remote control of devices, which is fast increasing with the advent of the internet of healthcare technology, might provide solutions whereby quick troubleshooting or assistance in calibration can be provided to the medical staff (user) remotely and easily.

ETHICS AND TECHNOLOGY ADOPTION

While the innovative sensing technologies have immense potential to revolutionize the healthcare system during a crisis such as the COVID-19, there are important ethical issues revolving around such technologies. Arguments concerning data confidentiality, ownership, and privacy are extremely challenging to address in a short duration of time, leading to a decrease in the adoption of the technology by community members.[311,312] Especially, the sensors which aim to provide immediate, detailed, and objective feedback of the measurement remotely to the information management systems must abide to the data protection legislatures of the given region. Even though passing the quality controls related to the data protection has nowadays become fast, the acceptability of these technologies in terms of their reliability remains low. Many medical staff still have concerns whether the developed technology has enough understanding of the biology to be properly informed from the measurement. Moreover, with traditional medicine, before a doctor provides a treatment, they must seek a patient's consent to reasonably inform them about the risks and benefits associated with the intended treatment. In this context, the risks and benefits of the mobile heath applications and self-use biosensors are not explicit as most often users are less willing to read the terms and conditions which usually pop up while using an application. However, toward the laboratory-based testing, biosensors and surface characterization tools eliminate ethical issues such as those associated with animal and human testing.[313,314] For example, if a drug is required to be developed in a short duration during a pandemic, large-scale testing can potentially be performed on high-throughput sensing platforms before it is considered viable to test on animals and humans. Large-scale testing could include a variety of characterizations to reveal molecular affinity, specificity, and cross reactivity with potential compounds inside the organism. Therefore, with the increased use of modern characterization tools and bio/chemical sensors, large uncertainties associated with quick drug development and testing can potentially be eliminated.

CONCLUSIONS

Biosensors and nanoscale visualization/characterization tools can be considered as innovative and promising tools that can lead to life saving decisions on treatment and a deep understanding of pandemic strains. As we have witnessed extreme challenges associated with the testing and validation of viruses in COVID-19, we need to be prepared in terms of diagnostics to prevent a spread of any new emerging infectious diseases. Recently, Narvaez *et al.* suggested seven main recommendations for biosensing community, including the funding agencies: (1) investment; (2) collaboration; (3) mass manufacturing; (4) devices should comply with regulation bodies such as FDA; (5) rigorously satisfy features such as disposability and low sample volume tests; (6) integrate with Internet Of Things (IOT), and (7) ease of use for members of the community.[315] With the rapid advancement of nanotechnology, we believe that the sensing mechanism will emerge with a multidisciplinary approach, to enable rapid and on-site detection of viruses for point-of-care diagnosis and prevent epidemics at an early stage.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

N.B. would like to thank support from Royal Society, grant number IEC\R3\193004 during the course of this work. Z.Y.

https://dx.doi.org/10.1021/acsnano.0c04421
ACS Nano 2020, 14, 7783−7807

thanks UK NERC fellowship grant (NE/R013349/2) and Royal Academy of Engineering Frontier Follow-up grant (FF \1920\1\36).

VOCABULARY

Soft-biosensor, Portable devices which utilize noncontact modes such as direct thermal imaging to detect biomolecules or disease signatures and subsequent analysis to provide the status of the disease using a customized software-based application right at the site of patient; **mHealth**, The practice of medicine and public health supported by mobile devices such as smartphones, tablets, personal digital assistants, and the wireless infrastructure; for example, contract tracing applications can be put in mHealth category of devices; **Wastewater-based epidemiology (WBE)**, A bio/chemical sensing approach for analysis of pollutants and biomarkers in raw wastewater to obtain both qualitative and quantitative data on the activity of all inhabitants within a given wastewater shed; **Serial femtosecond crystallography (SFX)**, A type of X-ray crystallography technique developed using X-ray free-electron lasers (XFELs) which includes multiple progress in sample delivery, data analysis, and collection; **Immune electron microscopy (IEM)**, Any method which uses molecules in interaction with antibodies in coincidence with electron microscopy to localize antigens or at the ultrastructural level; **Multifrequency AFM**, A collection of methods where the microcantilever is excited with a range of frequencies and corresponding signals at different frequencies are detected to be used as feedback parameters and information channels to explore the materials properties; **FD-based AFM**, The force−distance curves obtained from measuring the vertical force that the tip applies to the surface. This technique can be used to extract mechanical, chemical, and physical properties of nanomaterials and is the basis of probing the ligand−receptor bonds in physiological conditions

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https://dx.doi.org/10.1021/acsnano.0c04421
ACS Nano 2020, 14, 7783−7807