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Nano-assisted Detection of SARS-CoV-2: Recent Advances and Future Directions

Somayeh Jafarinejad*[1]†, Arafeh Bigdeli[2†], Angila Ataei-Pirkooh[3], Alireza Tabibzade [3], Alireza Ghasempour [4], Omid Purdakan[4], Parsa Panahi[5], Bita Mehravi[1,4]*

[1]*Finetech In Medicine Research Center, Iran. University of Medical Sciences, Tehran, Iran.*
[2]*Department of Chemistry, Sharif University of Technology, Tehran, 11155-9516, Iran.*
[3]*Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
[4]*Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Iran. University of Medical Sciences, Tehran, Iran.*
[5]*Student Research Committee, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
[†]*These authors contributed equally to this work.*

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ABSTRACT

In December 2019, an infectious respiratory disease caused by a new severe acute respiratory syndrome-related coronavirus (SARS-CoV-2), was reported in the city of Wuhan in China. Due to its fast expansion, the so-called "COVID-19" disease rapidly turned into a global pandemic and brought unprecedented challenges for the global community. Since its declaration by the world health organization (WHO) in February 2020, all healthcare professionals throughout the world have been trying to mitigate the spread of this virus and manage this disaster. Amongst various ongoing efforts, early detection of this virus and diagnosis of individuals and groups whom are infected with SARS-CoV-2 are pivotal steps that should be accomplished in the earliest possible moment. Nanostructures which are often used for targeting specific biological markers, can be considered as potential candidates for rapid detection of SARS-CoV-2, especially when fast, portable, easy-to-use, and accurate in-field detection kits are required. In this Review, we have summarized recent advances in the detection of coronaviruses in which nanostructures have been utilized to either generate or amplify the detection signal. Potential benefits of implementing nanostructures in the detection of SARS-CoV-2 has also been demonstrated. The applicability of coupling current detection methods with smartphone-based platforms, array-based sensing systems, wearable gadgets and other future directions of SARS-CoV-2 detection techniques have been further discussed.

1. Introduction

Coronaviruses are the causative agent of respiratory and enteric infections in human and animal hosts [1, 2] In human hosts, coronavirus infections range from mild and self-limited infections to severe pneumonia and bronchitis [1, 2]. Coronaviruses belong to a group of enveloped viruses, called the *coronaviridae* family, that possess a single-stranded positive-sense RNA genome. The length of the RNA genome in these viruses is about 26-32 kilo bases [1].Severe acute respiratory syndrome-related coronavirus (SARS-CoV) and Middle East respiratory syndrome-related coronavirus (MERS-CoV) [3, 4] which are in the same coronavirus family have been recognized to be highly pathogenic. The SARS-CoV was discovered from

* Corresponding author.; e-mail: mehravi.b@iums.ac.ir , somayeh.jafarinejad@gmail.com
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Guangdong, China in 2002-2003 [5] while the MERS-CoV, was initially originated in Saudi Arabia and Middle East in 2012 [6]. After almost one decade, in December 2019, pneumonia cases which their origin was not known, were seen in Wuhan, Hubei Province, China.

This pneumonia was caused by a novel coronavirus, named the SARS-CoV-2, due to its high genetic similarity with the SARS family[7-10]. In comparison to other known virus SARS-CoV-2 has about 50% similarity to the MERS-CoV , about 80% similarity to SARS-CoV and about 96% similarity to the bat coronavirus RaTG13[11, 12]. Since the expansion of this virus through the world has turned into a global epidemic, scientists and clinicians are searching for efficient methods to control and prevent its further spread. Generally, once viral infections appear, both on the basis of their function on the respiratory system and on the immune system, the first efforts for their detection, diagnosis and treatment are based on molecular techniques. polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are common molecular techniques to start with; however, due to their limitations advanced methods such as isothermal nucleic acids and amplification-based methods, might be employed. The complexity of viruses in terms of their latent periods as well as their molecular mechanism when affecting living organisms (such as humans) makes their detection challenging. Looking at Ebola, for example, when it was discovered in Africa in the early 1970s, it had a very short latent period and it was not easy to pass from one person to another, but in the early 21st century, patients with latent periods of more than two weeks were observed, disclosing that these types of viruses are becoming smarter for surviving against human nature and the environment[13-16]. Hence, when facing widespread viral infections there is an urgent need to develop rapid, sensitive, specific and low-cost detection methods, before the situation becomes severe (i.e., encountering probable virus mutations, uncontrollable spread rate, coexistence with other viral or bacterial infections). To date (April 27th, 2020), more than 2.9 million people have been infected and more than 207,000 have died from the results of the unprecedented growth of the virus worldwide, which confirms the necessity of more research in this field. Accurate detection methods as well as epidemic treatments can be then provided as soon as possible[17-19]. It seems that the approach which different societies have taken so far for this virus is similar to the approach they used for past epidemics, but they did not pay attention to a very important issue, and that is these type of viruses are becoming smarter and stronger day by day, and we may not be able to stop the virus with molecular approaches today. As demonstrated in figure 1, the SARS-CoV-2 virus contains four structural proteins of which three are on the surface of the virus and one is in the internal part. Matrix protein (M), spike surface glycoprotein (S) and small envelope protein (E) are presented on the surface and nucleocapsid protein (N) is presented inside the membrane of SARS-CoV-2, yet the only protein that is accessible via chemical and physical interactions of the outside matrix is the S protein, which is responsible for receptor-binding mechanisms as well[20-23]. Therefore, if nanotechnology wants to play a critical role in early diagnosis, detection or even treatment of the SARS-CoV-2, one of the approaches would certainly be targeting the S protein. In addition, the diameter of the virus is around 60 to 140 nm and it consists of a single-stranded positive sense RNA genome with the length of about 30k nucleotides. The genome encodes proteins from the RNA-Dependent RNA polymerase (RdRP) which are essential for the survival of the SARS-CoV-2 [24, 25]. In addition, a few research articles have presented the possible mechanism of SARS-CoV-2 on different cell lines including HeLa cells, and have shown that its interaction with the angiotensin converting enzyme 2 (ACE2) is responsible for the cellular entry mechanism of SARS-CoV-2 and maybe for the infectious mechanism.

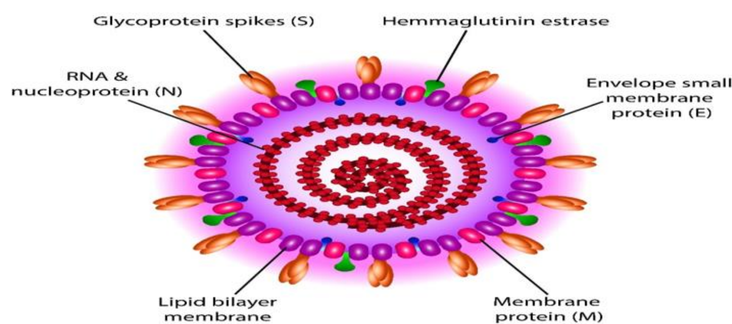

Figure 1. The structure of SARS-CoV-2 showing the structural M, N, E and S proteins.

Furthermore, some research groups have focused on designing a drug for inhibiting the interaction of ACE2 with SARS-CoV-2[26-28]. However, we believe that this approach might not be smart enough because these types of viruses are smart and would probably change their cellular entry mechanisms. The inhibition of ACE2 can be considered as a short-term and temporary solution. As clearly seen from the history of these types of viruses, if scientists could even use biological mechanisms for controlling the virus, they would unlikely be able to stop the next generation of these viruses, which will definitely come soon. Therefore, instead of focusing on these biological mechanisms, which we believe are more propagandistic, it would be better to focus on controlling the expansion cycle and creating these viruses using new technologies based on nano-concepts and/or even pico-technologies. In this review article, we have focused on different kinds of possible optical assays for the purpose of SARS-CoV-2 detection on different surfaces.

2. Current detection methods for SARS-CoV-2

Different nucleic acid amplification tests (NAAT) can be considered as sensitive tests for the detection of coronavirus [29]. For instance, reverse transcription PCR (RT-PCR), real-time reverse transcription PCR and reverse transcription loop-mediated isothermal amplification (RT-LAMP) can be mentioned as the most evaluated tests for coronaviruses [30-33]. On January 7th, 2020, the full genome sequence of the novel coronavirus named SARS-CoV-2 was available on Global Initiative on Sharing All Influenza Data (GISAID) [34]. Using new technologies speeds up the diagnosis methods especially in the case of the current pandemic with SARS-CoV-2 [35]. In the conducted study with Chan et al. the SARS-CoV-2 was firstly recognized by using full genome sequencing by oxford nanopore technology sequencing which led to further investigations in the field of molecular methods for this virus. The first conducted study in the field of SARS-CoV-2 diagnosis by Corman *et al*. [36], led to a detection method for this virus by real-time reverse-transcription polymerase chain reaction using RdRp, E and M regions as primer targets. Further investigations led to introducing a new digital PCR approach for the detection of this virus by Zhou and colleagues [37]. This digital PCR test showed a greater sensitivity than other common molecular methods and seems to be great for lower viral loads in clinical samples [37]. Recently, using pooled samples for real time PCR, a potential approach was presented for increasing the test capacity [38]. Furthermore, in a study by Casto and colleagues [39] an E gene based primer was suggested and the probe sets were the most sensitive for real time PCR assessment of SARS-CoV-2.Owing to the importance of the anti-body response as the public health perspective, some studies have focused on serological methods such as Enzyme linked immunosorbent assay (ELISA) [40]. Investigations on this virus have indicated that in the interdiction of its proteins during serological assays, the S protein is a greater target in comparison to the N protein[41]. Further studies have suggested the IgM level as a prognostic factor in COVID-19 patients [42]. Additionally, Huibin et al. [43] have suggested a cross reaction between SARS-CoV and SARS-CoV-2. In a relevant study, a low sensitivity has been achieved for rapid SARS-CoV-2 detection via immunochromatographic methods [44]. These studies reveal the importance of further detection methods for more accurate and faster approaches.WHO has released a list of standard primer and probe sets for the detection of the SARS-CoV-2 in COVID-19 patients. The list includes primer and probe sets from China CDC, US CDC, Institute Pasteur of Paris, National Institute of Infectious Diseases in Japan, Charité of Germany, HKU in Hong Kong SAR and National Institute of Health of Thailand [45]. A summary of the primer and probe sets is presented in Table 1 and four of the primary studies in the detection of SARS-CoV-2 is also listed [46-49]. Furthermore, WHO has introduced a reference website for all commercially available detection kits of SARS-CoV-2 in COVID-19 patients which can be found from the following link: "https://www.finddx.org/covid-19" [50].

Table 1. Summarized primer and probe set for SARS-CoV-2 detection

Author	method	Primer and probe sets	Target gene	Ref.
Chu	Real time PCR	5'-TGGGGYTTTACRGGTAACCT-3' (Y= C/T, R=A/G)	ORF1b	[47]
		5'-AACRCGCTTAACAAAGCACTC-3' (R=A/G)		
		5'-TAGTTGTGATGCWATCATGACTAG-3'(FAM/ZEN/IBFQ, W=A/T)		
		5'-TAATCAGACAAGGAACTGATTA-3'	N	

			5'-CGAAGGTGTGACTTCCATG-3' 5'-GCAAATTGTGCAATTTGCGG-3' (Probe FAM/ZEN/IBFQ)		
Chan	RT PCR		5'-CAAGTGGGGTAAGGCTAGACTTT-3' 5'-ACTTAGGATAATCCCAACCCAT-3'	RDRP	[51]
			5'-CCTACTAAATTAAATGATCTCTGCTTTACT-3' 5'-CAAGCTATAACGCAGCCTGTA-3'	S	
Chu	Real time PCR		5'- TCAGAATGCCAATCTCCCCAAC-3' 5'-AAAGGTCCACCCGATACATTGA-3' CY5-CTAGTTACACTAGCCATCCTTACTGC-3′BHQ1	E	[52]
China CDC	Real time PCR		5'-CCCTGTGGGTTTTACACTTAA-3' 5'-ACGATTGTGCATCAGCTGA-3' FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1	ORF1ab	[45]
			5'-GGGGAACTTCTCCTGCTAGAAT-3' 5'-CAGACATTTTGCTCTCAAGCTG-3' FAM-TTGCTGCTGCTTGACAGATT-TAMRA	N	
Institute Pasteur, Paris	Real time PCR		5'-ATGAGCTTAGTCCTGTTG-3' 5'-CTCCCTTTGTTGTGTTGT-3' Hex-AGATGTCTTGTGCTGCCGGTA-BHQ-1	RdRp	[45]
			5'- GGTAACTGGTATGATTTCG -3' 5'- CTGGTCAAGGTTAATATAGG-3' Fam-TCATACAAACCACGCCAGG-BHQ-1	RdRp	
			5'- ACAGGTACGTTAATAGTTAATAGCGT-3' 5'- ATATTGCAGCAGTACGCACACA-3' Fam-ACACTAGCCATCCTTACTGCGCTTCG-BHQ-1	E	
US CDC	Real time PCR		5'-GACCCCAAAATCAGCGAAAT-3' 5'-TCTGGTTACTGCCAGTTGAATCTG-3' FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ-1	N-1	[45]
			5'- TTACAAACATTGGCCGCAAA-3' 5'-GCGCGACATTCCGAAGAA-3' Fam-ACAATTTGCCCCCAGCGCTTCAG-BHQ-1	N-2	
			5'-GGGAGCCTTGAATACACCAAAA-3' 5'-TGTAGCACGATTGCAGCATTG-3' Fam-AYCACATTG GCACCCGCAATCCTG-BHQ-1	N-3	
			5'-AGATTTGGACCTGCGAGC G-3' 5'-GAGCGGCTGTCTCCACAAGT-3' Fam-TTCTGACCTGAA GGC TCTGCGCG-BHQ-1	Host RNAse P	
National Institute of Infectious Diseases, Japan (Nao et al [49])	Nested RT-PCR	First round	5'-TTCGGATGCTCGAACTGCACC-3' 5'-CTTTACCAGCACGTGCTAGAAGG-3'	ORF1a	[45]
		Second round	5'-CTCGAACTGCACCTCATGG-3' 5'-CAGAAGTTGTTATCGACATAGC-3'		
		First round	5'- TTGGCAAAATTCAAGACTCACTTT-3'	S	

			5'- TGTGGTTCATAAAAATTCCTTTGTG -3'		
		Seco nd round	5'-TCAAGACTCACTTTCTTCCAC-3'		
			5'-ATTTGAAACAAAGACACCTTCAC-3'		
	Real time PCR		5'- AAATTTTGGGGACCAGGAAC-3'	N	
			5'- TGGCAGCTGTGTAGGTCAAC-3'		
			FAM-ATGTCGCGCATTGGCATGGA-BHQ		
Charit é, Germany (Corm an et al [36])	Real time PCR		5'- GTGARATGGTCATGTGTGGCGG-3'	RdRp	[45]
			5'- CARATGTTAAASACACTATTAGCATA-3'		
			FAMCCAGGTGGWACRTCATCMGGTGATGCBBQ	Pan sarbeco	
			FAM-CAGGTGGAACCTCATCAGGAGATGCBBQ	SARS -CoV-2	
			5'-ACAGGTACGTTAATAGTTAATAGCGT-3'	E	
			5'-ATATTGCAGCAGTACGCACACA-3'		
			FAM-ACACTAGCCATCCTTACTGCGCTTCGBBQ		
HKU, Hong Kong SAR	Real time PCR		5'- TGGGGYTTTACRGGTAACCT-3'	ORF1 b	[45]
			5'- AACRCGCTTAACAAAGCACTC-3'		
			FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA		
			5'- TAATCAGACAAGGAACTGATTA-3'	N gene of SARS-CoV-2	
			5'- CGAAGGTGTGACTTCCATG-3'		
			FAM-GCAAATTGTGCAATTTGCGG-TAMRA		
Nation al Institute of Health, Thailand	Real time PCR		5'- CGTTTGGTGGACCCTCAGAT-3'	N	[45]
			5'- CCCCACTGCGTTCTCCATT-3'		
			FAM-CAACTGGCAGTAACCA-BQH1		

As mentioned earlier, several detection methods have been described for the detection of SARS-CoV-2 these days, based on molecular recognition. Implementing nanostructured materials, can assist in developing rapid and accurate test kits for virus detection. Nanostructures can either improve the performance of current molecular detection methods, or serve as new efficient recognition probes (Figure 2).

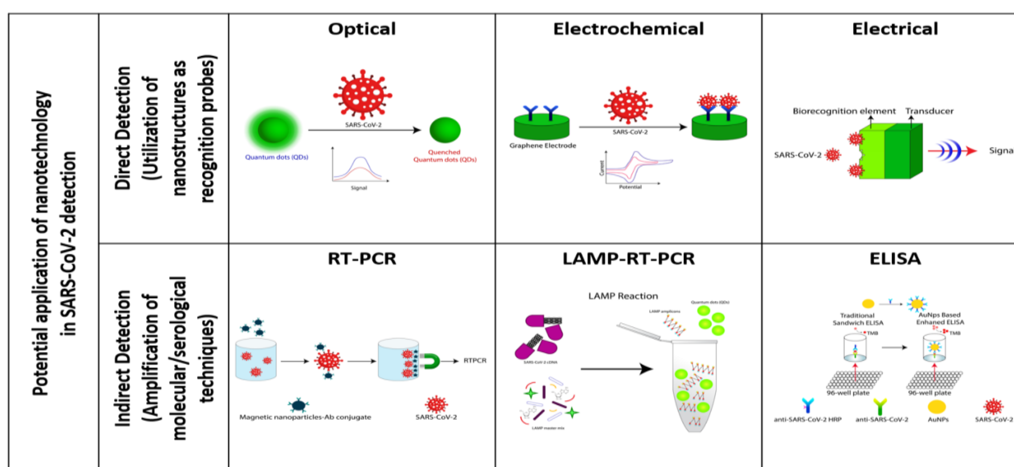

Figure 2. Schematic demonstration of the role of nanostructures in direct and indirect detection of SARS-CoV-2. Top: In direct routes, nanostructures are used as recognition probes to create quantitative signals (for instance: optical, electrochemical and electrical) relative to the presence of SARS-CoV-2 virus. Bottom: In indirect approaches, extraordinary characteristics of nanostructures are utilized to amplify signals in current virus detection methods such as RT-PCR, LAMP-RT-PCR and ELISA.

3.The role of nanostructures in SARS-CoV-2 detection

As in many other medical and biological applications in which the emergence of nanostructures has brought about noticeable enhancements, the use of nanostructures in viral detections has also led to outstanding improvements. Various detection strategies based on nanostructures have been presented in literature for rapid, sensitive and reliable identification of viral pathogens. As shown in figure 3, Gandhi et al.[53] reported an AuNPs-based LFIA for the detection of the SARS-CoV2 receptor-binding domain (RBD) that is antibody (Ab) tagged. In order to do, SARS-CoV2 RBD Ab was conjugated with AuNPs, acting as a detecting probe. A variety of factors, including membrane pore size, blocking circumstances, Ab coating concentration, and conjugate incubation, were taken into account when the manufactured LFIA strip was optimized. The best limit of detection for the improved LFIA strips was determined to be 1 ng/ml, which was supported by a smartphone app. The strips were verified in spiked buffer samples. In addition, when compared to the gold standard (RT-PCR), the AuNPs-LFIA strips efficiently identified RBD Ag in 100 clinical samples with 94.3% sensitivity and 90.9% specificity.

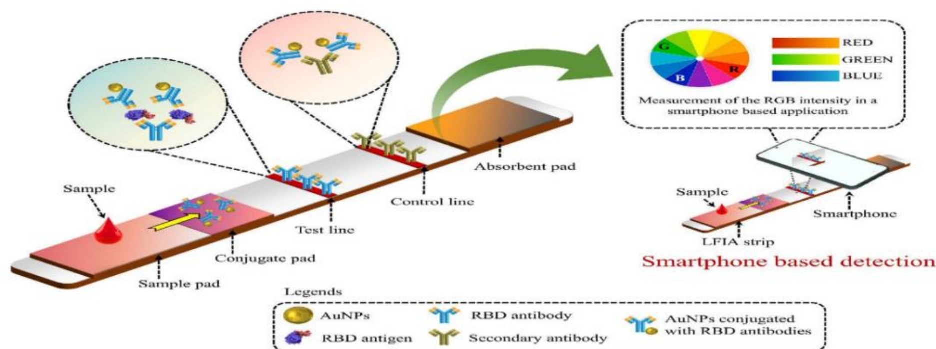

Figure 3. Created Lateral flow immunoassay system for SARS-CoV2 RBD protein detection in clinical samples and its color intensity analysis using smartphone application AuNP, gold nanoparticle; RBD, receptor-binding domain; RGB. [53]

Xu and his coworkers[54] presented an integrated active enrichment platform for the non-PCR direct hand-held detection of COVID-19 nucleic acid in nanoliter samples (Figure 4). The platform comprises a surface-enhanced Raman scattering (SERS) system based on a mobile phone, an integrated circuit system for ultrasonic output, and a capillary-assisted liquid-carrying device for sampling. Gold nanorods were chosen for biomedical applications due to their acoustic responsiveness and SERS-enhanced performance. Under ultrasonic aggregation, functionalized gold nanorods may efficiently collect and concentrate biomarkers. Such methods enable the very sensitive (6.15 1013 M) SERS detection of COVID-19 biomarkers in nanoliter (107 L) samples within 5 minutes, as well as the active assembly of gold nanorods in 12 s. they also confirmed the platform's potential for the detection of samples from throat swabs and showed off its great stability, reproducibility, and selectivity.

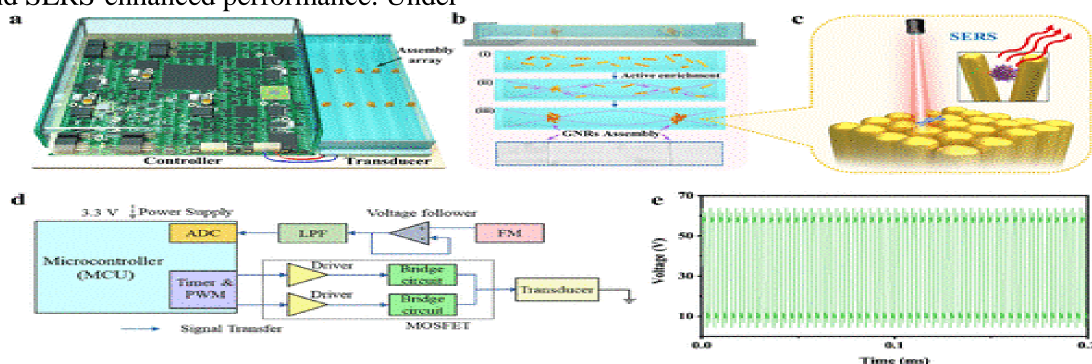

Figure 4. Concept for an integrated active enrichment platform with ultra-trace point-of-care biosensing. Diagrammatic representation of the fully integrated active enrichment platform may be found in (a). (b) Active enrichment in gold nanorod samples in nanoliter (10-7 L) volumes. Figure of gold nanorods collected for SERS detection in the assembly location is shown in (c). The driving signal generator and amplifier controller circuit is depicted in block diagram form in (d). (e) The steady control signal of the fully integrated enrichment platform[54] .

Also, Kim et al[55]. showed a nucleic acid biosensor platform that gets beyond these drawbacks by combining a novel nucleic acid amplification technique called nanoparticle-based surface localized amplification (nSLAM) with electrochemical detection (ECD). The system employs Fe3O4-Au core-shell nanoparticles with functionalized primers for nucleic acid amplification, which encourages the production of amplicons that accumulate on the nanoparticle surfaces and causes ECD currents to be significantly amplified, allowing the detection of target genetic material. The platform, when used with the COVID-19 model, has a remarkable sensitivity of 1 copy/L for 35 cycles of amplification, allowing the reduction of amplification cycles to 4 cycles (with a runtime of around 7 min), using 1 fM complementary DNA. Through direct polymerization, the nSLAM functions as an active promoter and participant in the nucleic acid amplification process. Through direct polymerization and binding of amplicons on the surfaces of the nanoparticles, the nSLAM functions as an accelerator that actively stimulates and participates in the nucleic acid amplification process. With the potential to be expanded to detect a larger range of biomolecules, this ultrasensitive fast-response technology is a promising tool for identifying newly emerging infections like the coronavirus.

Recently To accomplish quick and ultrasensitive detection, Zhang et al.[56] presented a SARS-CoV-2 detection sensor based on surface-enhanced Raman scattering (SERS). To achieve great specificity, the sensor used spike protein deoxyribonucleic acid aptamers as the recognition entity. The basis for the SERS experiment was a spherical cocktail of aptamers and gold nanoparticles (SCAP), and the SERS nanoprobe was an Au nanoparticle modified with a Raman reporter molecule that resonates with the excitation light. The SARS-CoV-2 S protein was targeted and captured using the SCAP substrate and SERS nanoprobes to create a sandwich structure on the Au film substrate, which may produce extremely powerful "hot spots" for ultrasensitive detection. By keeping an eye on variations in SERS peak intensity using a SCAP SERS substrate-based detection device, the SARS-CoV-2 S protein was analyzed. In less than 12 minutes, this test can detect S protein with a LOD of less than 0.7 fg mL-1 and pseudovirus at 0.8 TU mL-1. The findings of this study's simulated oropharyngeal swab technology suggested that it may be employed for clinical detection, offering a possible choice for quick and precisediagnosis and better management of SARS-CoV-2 transmission (Figure 5).

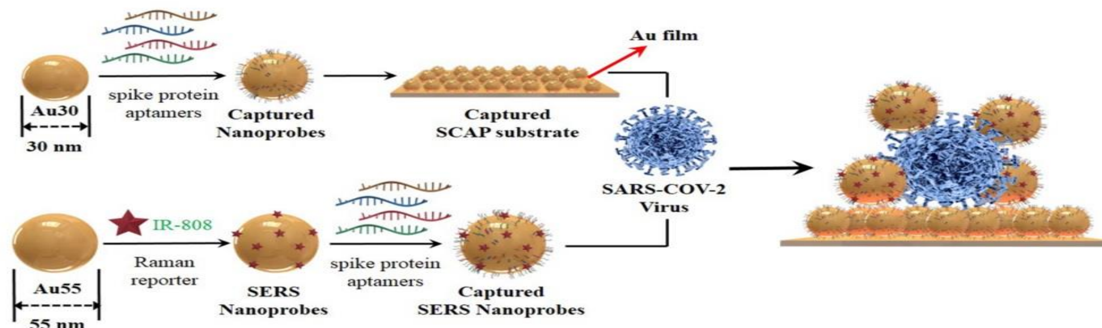

Figure 5. SARS-CoV-2 detection utilizing the SCAP SERS-based substrate: schematic and characterisation. (a) Schematic representation showing the sandwich structure's construction, the assembly of the SCAP substrate, and the capture-type SERS probe [56].

Paredes et al.[57] employed an electrochemical system for the detection of the nucleocapsid of SARS-CoV using gold nanoparticles. A monolayer of a thiolated oligonucleotide was immobilized onto disposable gold nanostructured screen-printed carbon electrodes. The detection was based on enzymatic amplification of the hybridization signals which led to highly sensitive detection of the target sequence. The in situ formed gold nanoparticles acted as an immobilization and transduction surface and greatly improved the sensitivity. In another work, taking advantage of the presence of gold nanoparticles in an electrochemical immunosensor, Eissa et al.[58]designed a multiplexed detection platform for the coronavirus family. The virus detection of this immunosensor was based on a competitive assay which was carried out on an array of carbon electrodes modified with gold nanoparticles. As depicted in Figure 6, the biomarkers of MERS-Cov viruses were immobilized on the surfaces of the electrodes and competed with the free viruses in the sample in binding to the added antibodies. The binding of the antibody to the immobilized protein resulted in a decrease in the electrochemical peak current

which was attributed to the coverage of the electrode surface with the bulky sized antibodies. Moreover, Chen et al.[50] presented the ability of Ag nanoparticle labels for specific detection of H1N1 infulanza virus. A polyclonal antibody was labeled by silver nanoparticles and was then incubated in the microplate to consruct sandwich complexes. The addition of an acidic solution caused Ag conjugates to release into the solution which led to a luminol chemilominescent signal enhancement. A number of reports also exist on the use of CuO nanoparicles[59] for signal amplification in the detection of H1N1 virus. Table 1 summarizes a list of recent reports on the application of various types of nanoparticles in the detection of corona viruses. The main components of the probe along with specific targets and the assay type has been demonstrated for each of the probes reported for SARS, MERS and other viruses in the corona family.

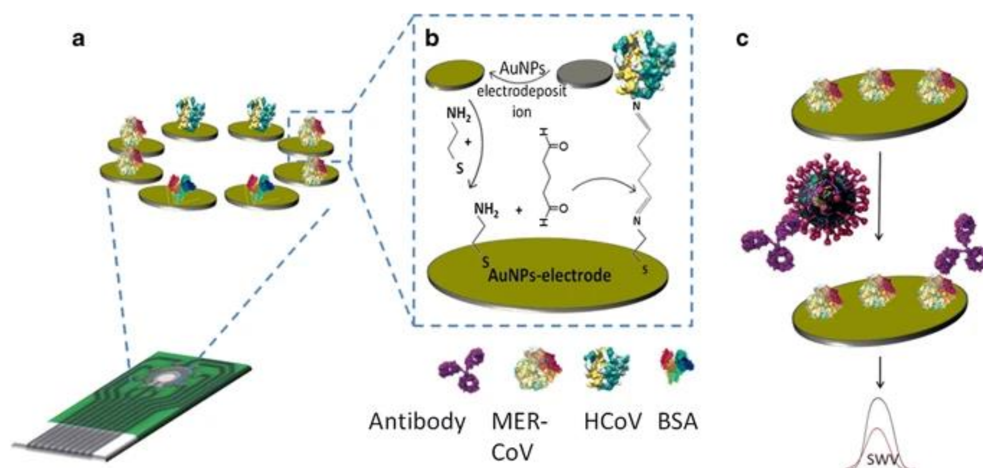

Figure 6. (a) Schematic illustration of the electrochemical immunosensor array, (b) immobilization of virus biomarkers on carbon electrodes modified with gold nanoparticles, (c) the competitive detection process [58].

Table 2. Summary of coronavirus detections based on nanostructures.

Virus es	Target	Probes	Biomolec ules	Detection Methods	eferenc es
MER S-CoV	E protein gene (upE) And (ORF) 1a	gold nanoparticles	dsDNA	Colorimetric	60]
MER S-CoV	6G (R6G) and carboxytetramethylrhoda mine (TAMRA)	magnetic beads	DNA	PCR-based test	61]
SAR S	Polyadenine-Modulated DNA	gold nanostars (AuNS)	DNA	-	62]
MER S-CoV, MTB, and HPV	Oligonucleotide s	AgNPs	DNA	Colorimetric	63]
SAR S	oligonucleotide strands	Gold Nanostructured	DNA	Electrochemica l	57]
MER S-CoV / HCoV	antigen	gold nanoparticle	antibody	Electrochemica l	58]
MER S-CoV	protease	papain-like protease (PLpro) and the 3-chymotrypsin-like protease (3CLpro	-	Biosensors	64]
SAR S	antigen	-	monoclon al antibodies b1 and h12	label-free immunological method	65]
SAR S	SARS biomarker N protein	In2O3 Nanowire	Antibody mimic proteins (AMPs)	nanowire biosensor	66]
SAR S-CoV	anti- SARS-CoV N protein (anti-N protein I)	polymethyl methacrylate (PMMA)	SARS-CoV N protein	fiber-optic biosensor	

SARS	nucleocapsid protein	aptamer	ssDNA	ELISA	67]
SARS	antigen	-	antibody	Piezoelectric Immunosensor	68]
SARS	nucleocapsid protein	quantum dots-conjugated RNA aptamer	RNA	-	69]
SARS-CoV-2	FITC / DIG	Dye streptavidin coated polymer nanoparticles	Anti FITC / Anti DIG	RT-LAMP coupled with nanoparticles-based biosensor	70]
feline coronavirus (FCoV)	fAPN	BHK-liposome	-	single particle tracking	45]
SARS-CoV-2	ORFlab and N gene	Magnetic nanoparticles	monoclonal anti-DENV2 NS1 antibodies	Flow-cytometry	71]
SARS	29 751-base genome of the SARS	carboxyl groups coated magnetic nanoparticles	RNA	RT-PCR	71]
SARS	N protein	Gold nanoparticle	Sialic acid	Colorimetric	72]
SARS	DNA	CdSe/CdS/ZnS Quantum Dot	RNA	Fluorometric	73]
SARS-CoV-2	S protein	Magnetic nanoparticles	-	Magnetic response	74]
SARS-CoV-2	COVID-19 antigen	Gold nanoparticle	Anti-spike protein	Colorimetric	75]
SARS-CoV-2	DNA	Gold nanoparticle	-	Raman spectroscopy	76]
SARS-CoV-2	COVID-19 antigen	Gold nanoparticle	DNase activity of Cas12a	Colorimetric	77]

In 2004, a colorimetric approach was presented for SARS-CoV detection by Li and co-workers[78] in which spherical gold nanoparticles were used for the detection of the SARS-CoV encoded poly protein by ORF1a/b gene [78]. Meanwhile, gold nanoparticles have been reported as optical detectors in some different virus detection approaches. For instance, Azzazy et al.[79] used a new strategy based on the aggregation of gold nanoparticles in the presence of HCV RNA in biological fluids. In this simple, rapid and sensitive assay which did not require RNA amplification, the complementary primer played as a stabilizer agent for gold nanoparticles. In the presence of the complementary target RNA, the formation of double strands inhibited the adsorption of the primer onto gold nanoparticles, as the stabilizer. As a result, gold nanoparticles aggregated and the solution color change from red to blue.

The variety in the above-mentioned nanostructure-based corona virus probes implies the potential of nanostructures to be extended for the design and construction of efficient sensors for SARS-CoV-2, as it belongs to the same virus family. Tunable characteristics of nanoparticles allow engineering different types of nanostructures with desirable properties, including their size, shape, optical and magnetic properties and so on. The surface of nanoparticles can be functionalized to finely attach to a target of interest, allowing the design of highly sensitive and specific probes.

Employing nanoparticles can improve the limit of detection of current SARS-CoV-2 probes, making early detection of this virus feasible. Nanoparticles not only can assist in the design of new nanoparticle-based probes, but can also efficiently improve lengthy PCR-based detections and save a huge amount of time, which is a critical issue in the management of such a fast-outbreaking virus. Compared to molecular techniques, which require sophisticated instrumentation and can only be performed by laboratory clinicians, the design of user-friendly test kits for accurate detection of SARS-CoV-2 are highly preferred, especially when affordable and accessible to all. Figure 7 outlines some possible advantages of nanostructure-based detection methods for SARS-CoV-2.

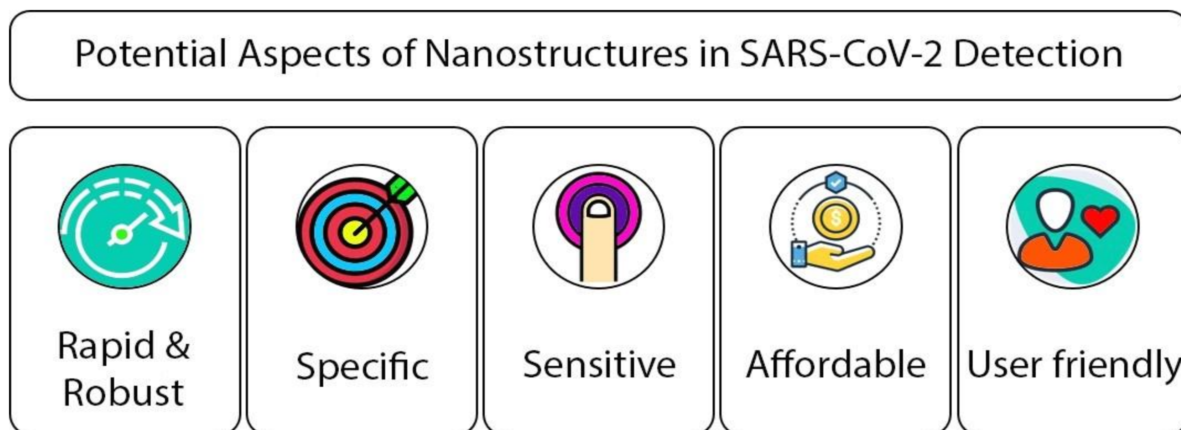

Figure 7. Potential advantages of nanostructures in SARS-CoV-2 detection.

In the following, recent reports in which nanoparticles have been employed for the development of SARS-CoV-2 detection have been presented. As previously mentioned, RT PCR assay is a common method for SARS-CoV-2 detection. However, it has some drawbacks which result in low precision and accuracy and false negative results, mainly caused by low RNA extraction efficiency[80]. Therefore, high purity extraction from samples is the most critical point to meet for well-organized RT-PCR assay. Chinese researchers[81] have demonstrated an alternative RNA extraction technique instead of the commonly used methods. They have applied the high-throughput efficiency of carboxyl polymer-coated magnetic nanoparticles (MNPs) in the absorption of RNA molecules as a new approach for RNA extraction. In this strategy, the virus lysis and RNA binding steps are conducted as one phase and produce RNA-MNPs. This compound can be added to RT-PCR reactions directly lowering the required time as well as simplifying/facilitating the protocol. Eliminating toxic reagents, achieving high purity and productivity and having compatibility with various isothermal amplification methods such as the LAMP method are other important advantages of this new RNA extraction method.

Recently, isothermal amplification techniques such as LAMP have been used as highly specific methods for virus detection. The amplified DNA is detected by three different approaches consisting of turbidity, colorimetry and fluorometry. Various dyes such as Acridine Orange, Hydroxynaphthol Blue (HNB) and Propidium Iodide are used for naked eye observation of color changes in the presence of viruses. Fascinating optical properties of nanostructures demonstrate their capability to be used as colorimetric or fluorometric probes. Researchers have tried to improve these techniques with different strategies such as employment of the quantum dot barcode technology[21]. Kim et al,[82] have shown that the use of quantum dot barcodes in isothermal amplification methods can be very useful for diagnosing patients with hepatitis B. Chee et al.[83] have reported the combination of the LAMP technique with the reverse transcription assay (RT-LAMP) for identification of several pathogens, including influenza viruses and SARS virus families. According to these investigations, RT-LAMP technique has a potential to be used for SARS COV-2 virus detection. Wang et al.[70] combined RT-LAMP with nanoparticle-based biosensors to set up a rapid and precise method for simultaneous detection of two target genes of SARS-CoV-2 in a one-tube reaction. The designed nanobiosensor consisted of four components: a sample pad, a conjugate pad, a detection region and an absorbent pad. The detection pad contained immobilized anti-fluorescein antibody, anti-digoxigenin antibody and biotinylated bovine serum albumin, while dye and streptavidin coated polymer nanoparticles were immobilized on the conjugate region. Patients specimens, after cell lysis and RNA extraction and finally RT-LAMP process, were added to the nanobiosensor device and each receptor interacted with its specific ligand. The appearance of colorful lines in the detection pad indicates a positive result of SARS-CoV-2.

4. Conclusion

The end of 2019 and the beginning of 2020 was accompanied with the appearance of a global pandemic caused by SARS-CoV-2. The high outbreak of this virus was challenging to current detection methods and pushed scientists around the world to develop fast detection kits with a minimum response time, low enough to beat the

spread rate. In the meantime, the proposed detection methods should also meet sensitivity concerns and be reliable, avoiding false positive and false negative results. In this review, we have summarized current detection methods which have been used for the detection of viral infections regarding the corona virus family. The application of nanostructures and potential benefits of utilizing nanostructures in this field has been demonstrated.

5. Future outlook

Despite the growing number of publications on nanostructure-based detection methods for corona virus family, there is a continuing need to improve current virus detection techniques because of the high expansion rate of COVID-19. Most of the methods reported so far, have focused on single detection of each of the viruses of this family, such as MERS or SARS. Designing a detection platform for multi-virus detection will be of great interest. As announced by WHO, the symptoms of SARS-CoV-2 infection such as fever, cough, tiredness and shortness of breath are similar to the influenzas; thus, the development of multi-virus detection sensors based on nanoparticles capable to discriminate among viruses belonging to the same family could be very useful. Such array-based nano sensors seem to be ideal for this sensing situation because they not only allow multi-analyte testing, but can also be available, facile, portable and cost-effective. Furthermore, as with many other probes, they can be coupled to smart phones for on-site easy-to-use detections. Suitable modules can be coupled to these smart-phone based detection kits such as light sources with desired wavelengths, RGB or other signal readouts, data analysis packages for signal processing or pattern recognition, and other components that facilitate the application of such sensors. Moreover, implementation of nanostructures in wearable sensors is another potential application of nanoparticles in the near future for fast detection of SARS-CoV-2. Alterations in body temperature and lung function as well as analysis of body fluid can be screened and accomplished very well by these sensors. In fact, simultaneous monitoring of body temperature or lung function with multi-parameter wearable sensors may allow the identification of an infected person in early stages who has not shown any signs of this viral disease yet. Among the approaches discussed herein, the focus of current research is mainly on reducing the response time of SARS-CoV-2 detection kits and overcoming the current time-consuming and laborious detection techniques. Owing to the high outbreak rate of COVID-19, designing sensitive detection kits with very low response times can dramatically prevent the further spread of SARS-CoV-2 infection.

Vocabulary

Pandemy: an outbreak of a disease that occurs over a wide geographic area and affects an exceptionally high proportion of the population.

RT-PCR: Reverse transcription-polymerase chain reaction, is a modification of conventional PCR, whereby RNA molecules are first converted into complementary DNA molecules that can then be amplified by PCR.

LAMP: Loop-mediated isothermal amplification is a one-step isothermal gene amplification method that uses multiple primers for creating continuous loop structures during DNA amplification.

COVID-19: is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Nanobiosensor: diagnostic devices with nanoscale dimension used for the detection and measurement of biomolecules and cells

ELISA: Enzyme-linked immunosorbent assay is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones.

Nanostructures: Refer to materials or structures that have at least one dimension between 1 and 100 nm.

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