Electrochemical investigations for COVID-19 detection-A comparison with other viral detection methods

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ABSTRACT

Virus-induced infection such as SARS-CoV-2 is a serious threat to human health and the economic setback of the world. Continued advances in the development of technologies are required before the viruses undergo mutation. The low concentration of viruses in environmental samples makes the detection extremely challenging; simple, accurate and rapid detection methods are in urgent need. Of all the analytical techniques, electrochemical methods have the established capabilities to address the issues. Particularly, the integration of nanotechnology would allow miniature devices to be made available at the point-of-care. This review outlines the capabilities of electrochemical methods in conjunction with nanotechnology for the detection of SARS-CoV-2. Future directions and challenges of the electrochemical biosensors for pathogen detection are covered including wearable and conformal biosensors, detection of plant pathogens, multiplexed detection, and reusable biosensors for on-site monitoring, thereby providing low-cost and disposable biosensors.

1. Introduction

Viruses are the simple structures consisting of genetic information that can replicate through the hosts [1]. These intercellular agents cannot replicate outside the host cell, but remain as a crystalline structure for longer period until they come in contact with a host [2]. The genetic material, either DNA or RNA, is surrounded by a sheet of protein, called capsid. Once the viral genome enters the host cell, the replication and protein synthesis machinery is hijacked to make more virus particles, called Virions [1] that have the ability to infect new cells after releasing from the host cell. Viruses get easily adapted to new conditions due to mutation, thereby increasing the genetic diversity. Since virus do not have any enzyme system, antibodies may not affect them. These viruses consist of only digestive enzyme helpful to dissolve the host cell membrane and viruses belong to a wide variety of families such as DNA virus and RNA virus. Typical examples are *Adenoviridae, Parvoviridae, Herpesviridae, Retroviridae, Rhabdoviridae,* etc [3].

Recently, virus belonging to a big family of *Coronaviridae* has

significantly raised concerns to the mankind, which can cause mild condition such as common cold to serious respiratory disorders [4]. The first human corona virus was characterised during the sixties, which was obtained from the respiratory tract of an adult suffering from a common cold [5] and since 2003, five new human coronaviruses have been identified. These have been divided into four sub-groups, called alpha, beta, gamma, and delta. Alpha and beta are prone to infecting the mammals, whereas gamma and delta strains are more common in birds [6]. The most four common once are 229E, NL63, OC43 and HKU1 and the three less common ones are MERS-CoV, a beta virus that has caused Middle East respiratory syndrome (MERS), SARS-CoV, a beta virus caused severe acute respiratory syndrome (SARS), SARS-CoV-2 may be the cause for COVID-19 [7].

Since December 2019, Wuhan city in China witnessed the spread of a novel corona virus, called SARS-CoV-2. This virus was clinically characterised by the respiratory syndrome having an effect ranging from mild respiratory illness to severe acute respiratory distress syndrome [8]. At present, the virus has moved out of China and spread around the world, severely affecting many nations including the United States of America, United Kingdom, Russia, Spain, and Italy infecting a total of 3,57,38,553 people by October 06, 2020 [9]. The World Health Organisation (WHO) has estimated the reproductive number (R_0) of novel infection in the range of 2 to 2.5, which is higher than SARS (1.7–1.9) and MERS (less than1), signifying an intense pandemic potential of SARS-CoV-2 [10].

2. Virus characteristics and its symptoms

The structure of corona resembles roughly a spherical shape with projections appearing like a crown (Fig. 1). The virus has approximately 125 nm size consisting of an envelope of 85 nm dia, while the spikes are 20 nm long. It is a single stranded RNA with the size ranging from 26,000 to 37,000 bases and is the largest known genome among the RNA viruses [11]. Envelope in the virus is made of lipid bilayer, which is anchored with membrane proteins, envelope proteins and spike structural proteins in the ratio of 1:20:300. The spike protein in corona virus is a class I fusion protein. With two subunits S1 and S2, the S1 subunit is characterised by two subdomains, viz., C-terminal and N-terminals, which are capable of binding receptors angiotensin-converting enzyme 2 (ACE2). The spike protein is heavily glycosylated and facilitates the attachment to host receptors by accessing ER with the help of N-terminal signal sequence. The nucleocapsid protein is heavily phosphorylated that binds to RNA *in vitro*. This protein is helpful in packaging the encapsulated genome into the viral particles by roping the viral genome to replicase-transcriptase complex. The most abundant structural protein in virus is the membrane protein, which has two different conformations that can promote the binding to nucleocapsid. In small quantity,
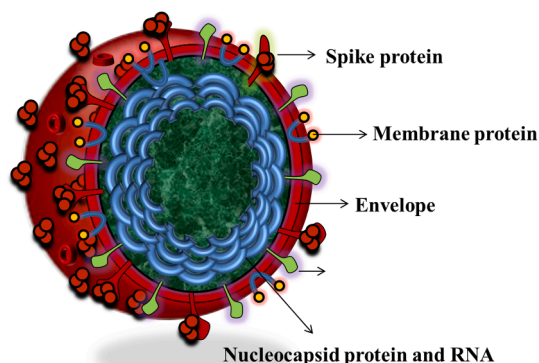

Fig. 1. Structure of SARS-CoV-2.

the envelope protein acts like a Trans membrane protein for ion channel activity [12,13].

In general, the mechanism of infection can be understood as: the attachment to host receptors takes place with spike proteins; the genomic binding to replication-transcription complex is abetted by nucleoplasid proteins, and finally, the membrane protein and envelopes protein provide the shape to virion particles and the release of particles.

The basic outline of transmission of the virus is due to person-to-person contact and the respiratory virus can be transmitted via respiratory droplets when an infected person coughs or sneezes. As per the guidelines of Centers for Disease Control and Prevention (CDC), maintaining a distance of 6 feet between the people would work more effectively as the gravity would otherwise make the droplets of infected person to fall on the ground within 6 feet [14]. However, there is another recommendation that 6 feet rule is out-dated and is based on the studies between 1930 s and 1940 s. The respiratory droplets may travel a fair distance beyond 6 feet depending on the airflow conditions [15,16]. The other mode of transmission would be by contact transmission and investigations have suggested that the virus is viable from 3 h in air to 72 h on plastic and stainless steel [17]. In addition, the blood and stool of the infected person contains SARS-CoV-2 RNA even though it is still unknown that any exposure to non-respiratory body fluids may cause the infection [18].

WHO has suggested that if a person is infected by SARS-CoV-2, it would normally take five to six days for the first symptom to appear [19]. The incubation period of virus may vary based on individual cases lasting up to 40 days and 14 days as the median incubation period [20]. In addition, the incubation period may show variations based on the age and immune response of the person. It has been reported that persons aged below 70 years have a longer period of incubation (20 days) compared to persons above 70 years (11.5 days) [20]. The symptoms include cold and influenza, which may vary from mild to severe, leading to death depending on the previous underlying diseases such as heart, diabetes, lungs, etc. A recent report by the WHO on the symptoms of SARS-CoV-2, tested on more than 70,000 cases in China, are that fever observed in 88% of cases; dry cough and sore throat about 68% fatigue and diarrhoea about 38% and 4%, respectively [21]. Presence of asymptomatic carries of the virus also requires high priority attention [22].

Studies have suggested that nearly 20% of the infected people had co-morbidities with respect to dysfunction of other organs including renal impairment. Patients with underlying cardiovascular disease frequently demonstrate co-morbid heart failure [23]. The diagnosis difficulty increases as the symptoms develop from initial symptoms in cardiovascular system, digestive system and nervous system. Further, serious cases may develop into acute respiratory disease syndrome, irreformable metabolic acidosis, septic shock and multiple organ failure. Nearly 80% of the infected people show decrease in the WBC count and lymphocytopenia observed in 72.3% [24]. The chest computed tomography images have shown that lung is involved in almost all cases showing the lesions in multiple lung lobes. Current case studies have suggested that majority of the patients have a good recovery rate, but poor recovery rate has been observed in aged persons, especially those with underlying chronic diseases.

3. Detection methods

Early detections of SARS-CoV-2 are very useful in the initial diagnosis and this will be quite valuable in controlling the spread of the infection. Hence, a significant approach towards rapid and accurate detection of virus is necessary. Several methods used for the detection of SARS-CoV-2 are the most commonly used ones that are either molecular tests or serological tests. The viral or molecular tests often indicate the active infection and these are designed to detect the genetic material of the virus. On the other hand, serological tests can detect the antibodies present in the blood and tissues produced during the fight against the

virus, but these tests do not show about the current infected state.

Recent developments in molecular biotechnology have facilitated nucleic acid detection methods that are growing rapidly as the revolutionary technology, since polymerase chain reaction-based methods are advantageous to provide high sensitivity and rapid detection. Further, non-PCR-based methods are developed for the detection of SARS-CoV-2 RNA, which involves nucleic acid sequence-based amplification and isothermal nucleic acid amplification.

3.1. Polymerase chain reaction-based methods

PCR is a vital tool used in molecular biology to make millions to billions of DNA copies rapidly. It is very much advantageous to the medical fraternity that uses a small sample of DNA and amplifies it to significant amount for detailed investigations. This process involves initially separating a DNA strand containing the gene segment and a primer can be used to mark its location. Further, DNA polymerase accumulates a copy to each separated strand and then copies the copy continuously. The advantages and wide range of applications of PCR–based technique can be routinely and reliably used for detecting SARS-CoV-2 [25,26]. Since SARS-CoV-2 consists of RNA as a genetic material, a reverse transcription is also carried before the PCR followed by product determination using appropriate detection methods or an instrumental analysis, which includes sequencing or gel visualization [27,28]. Diagnosis in the early infection stage is more helpful and hence, real-time reverse transcriptase-PCR is predominantly used than the conventional PCR assay [29].

Van Elden et al. [30] described some disadvantages of RT-PCR methods such as contamination, time consumption, sample handling and analysis of post PCR can be easily avoided using TaqMan-based real-time RT-PCR. The sensitivity of this method was further improved by Yip et al. [31] using two TaqMan probes as a replacement for one probe for the detection of SARS-CoV. However, massive efforts are needed to overcome the difficulties in clinical detection such as lack of safe and external positive controls (EPC). EPC is an important component, which when the problem was avoided by using the armoured RNA as EPC for the detection of SARS-CoV [32] having the low detection limit of 10 copies /µL. Further, significant consideration should be given to reduce the risk of false negative results due to the variation in genome sequence due to genetic diversion caused as a result of rapid mutation in corona viruses. In such cases, multiplex RT-PCR is favourable to detect via multi-targeting detection of the coronavirus. Distinguishing between the non-pathogenic and pathogenic strains by using the mismatch-tolerant molecular beacons [33] demonstrated the detection limit of 5 copies/ reaction [33].

3.2. Non-polymerase chain reaction-based methods

PCR-based techniques are widely used, but these techniques require the separation of DNA strands using a thermocycling, which limits its application in actual field applications. Isothermal nucleic acid amplification-based (INAA) methods developed in the past two decades without using thermocycler machine are useful for the detection of nucleic acid target sequence.

Loop-Mediated Isothermal Amplification (LAMP) is one of the INAA method with higher efficiency commonly used for the amplification of DNAs and RNAs exhibiting higher sensitivity and specificity (Fig. 2). This method involves the use of a DNA polymerase along with four sets of specially designed primers that identifies six distinct sequences on the targeted DNA (Fig. 3) [34]. Gel electrophoresis is another commonly used approach to analyse the amplified products after LAMP essay. Poon et al. [35] and Pyrc et al. [36] demonstrated a LAMP reaction for SARS-CoV with the detection rates similar to those of the conventional PCR-based methods and 1 copy of RNA template per reaction, respectively.

The problem of virus detection can be simplified by using the precipitation of magnesium pyrophosphate or fluorescence dyes monitoring
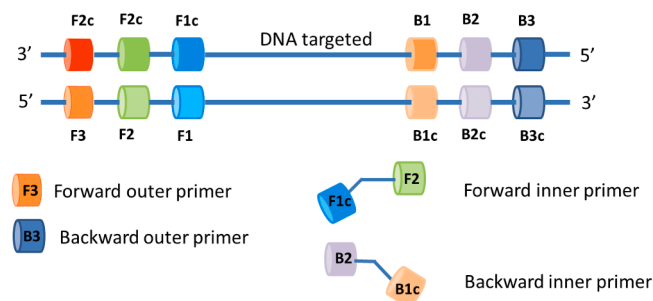

Fig. 2. Components for loop mediated isothermal amplification.

turbidity [37]. Shirato et al. [38] demonstrated one such a procedure for the detection of MERS-CoV RNA with the capability of detecting 3.4 copies without cross reaction with other respiratory viruses. Further, Thai et al. [39] demonstrated photometric method of measuring turbidity in one-step single-tube accelerated real-time quantitative for SARS-CoV having the sensitivity of 100-folds more than that of the conventional RT-PCR with a detection limit of 0.01 plaque formation unit.

In the above mentioned methods, the problems aroused due to the fact that primer dimer or non-primer reactions cannot be excluded as these techniques rely on nonspecific signal transducers such as solution turbidity or fluorescence dye intercalation. In such situations, sequence-specific LAMP-based methods that can readily separate nonspecific noise with true signal may be advantageous. In this direction, Huang et al. [40] proposed a method using RT-LAMP-VF (RT-LAMP and a vertical flow visualization strip) for the detection of MERS-CoV with a detection limit of 10 copies/µL. However, the major contributions from Ellington's group improved in terms of specificity, reliability and LAMP detections. Replacement of dye in fluorescence detection with toehold-mediated strand exchange reaction for RT-LAMP-VF showed the detection of 0.02 to 0.2 PFU MERS-CoV without cross reaction with other respiratory viruses [41]. Du et al. [42] demonstrated a method combining strand exchange signal transduction, LAMP and a glucometer for the detection of MERS-CoV with a sensitivity of 20–100 copies/µl, equating to atto-molar.

Rolling circle amplification (RCA) is another technique that has attracted various scientific groups in nucleic acid determination. It is a unidirectional process of replicating nucleic acid producing numerous copies of circular molecules of DNA and RNA capable of 109 folds of amplification of each circle in 90 min under isothermal conditions. Further, the use of RCA has advantages such as requirement of minimum reagents and exclusion of false-positive results, which are frequently observed in PCR-based methods. An efficient method for sensitive detection of SARS-CoV genome using RCA in both liquid and solid phases was proposed by Wang et al. [43].

3.3. Microarray based methods

A typical microarray experiment involves the hybridization of an mRNA molecule to DNA template from which it is originated. Many DNA samples are used here to construct an array. The amount of mRNA bound to each site on the array indicates the expression level of various genes and this number may run in thousands. All the data are collected and a profile is generated for gene expression in the cell.

Shi et al. [44] designed 30 specific 60 mer oligonucleotide microarray based on TOR2 sequence in clinical samples that was successfully used for detecting SARS coronavirus. These designed microarrays represent the whole genome of SARS coronavirus, which was printed into an oligo microarray, and then applied to hybridizing with the samples of SARS patients treated and labelled by RD-PCR. This method offered results for seven samples hybridized on the microarray with no signals on blank and negative probe sites. Rapid mutation in SARS-CoV
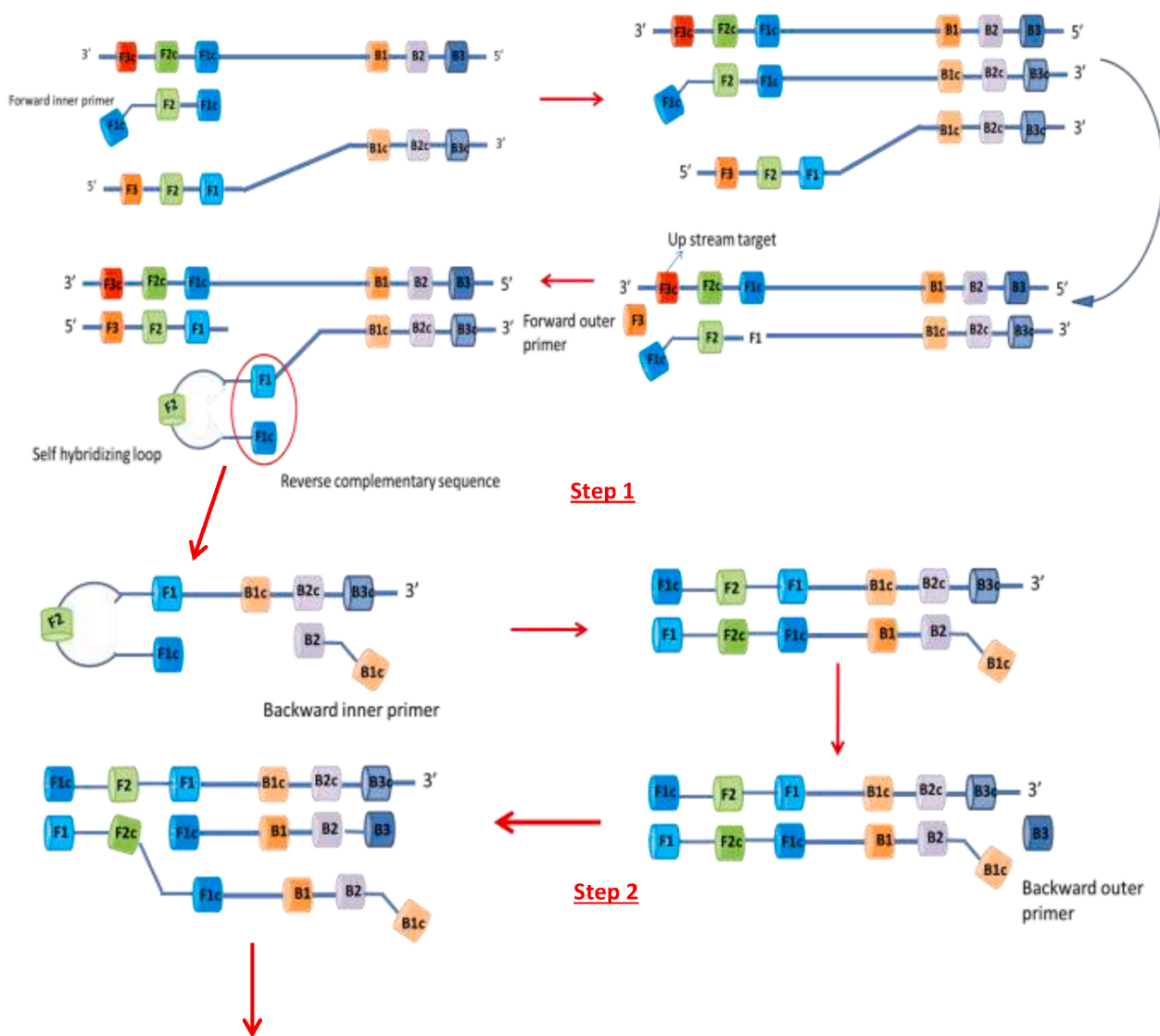
Fig. 3. Schematic presentation of loop mediated isothermal amplification process.

associated with 27 single nucleotide polymorphism mutations among the spike gene has lead to epidemicity. Considering such a mutation problem in SARS-CoV, Guo et al. [45] designed a microarray based on a single nucleotide polymorphism DNA, which would detect and genotype these single nucleotide polymorphisms and allow us to understand the pathogenicity and epidemicity of a given strain. Amplified products of cDNA from PCR technique of different strains of SARS-CoV were hybridized on the fabricated microarray. This method detected 24 single nucleotide polymorphism and the method was helpful to identify the strain with 100% accuracy for 19 samples in detecting and genotyping.

The sudden outbreak of viruses as observed in case of SARS-CoV 2 allowed us to think that the designed diagnostic assay should be able to be used at or near the point-of-care (POC) to detect a wide range of strains. Luna et al. [46] developed a non-fluorescent method for detecting the entire coronavirus genus with a detection limit of 15.7 copies/reaction and 100 copies/sample in patients with severe acute respiratory syndrome. Hardick et al. [47] evaluated a novel, portable, and near-POC diagnostic platform based on the microarray chip, the Mobile Analysis Platform (MAP), which showed a good performance in identifying the virus within an acceptable detection limit.

4. Current scenario in the detection of viruses

Electrochemical investigations favour significant advantages such as simplicity in design, higher sensitivity and selectivity, low cost equipment, lower power need and easy to integrate within the microfluidic devices compared to other proposed methods [48-50]. Electrochemical investigations have also demonstrated excellent applications in health care applications [51,52]. Recently, the World has witnessed the outbreaks of diseases associated with viruses such as Ebola, MERS-CoV, SARS-CoV-1 and SARS-CoV-2 highlighting the need for rapid testing kit that can be used in the community to avoid further pandemic. A novel device with advanced instrumentation for cases such as COVID-19 is an upcoming challenge for the point-of-care diagnostic industries. However, it was observed that OECD countries have achieved a massive scaling in testing of the coronavirus that are predominately based on PCR-centralised laboratory testing than using the point-of-care devices. Moreover, reducing the sample-to-answer time is also crucial in contact tracing. Thus, reliable and high throughput testing devices continue to play the central role in containing the pandemic. Hence, in this section, we will discuss the advances made in electrochemical techniques for the detection of pathogen that can be reliable and powerful to fight against even for any future pandemics.
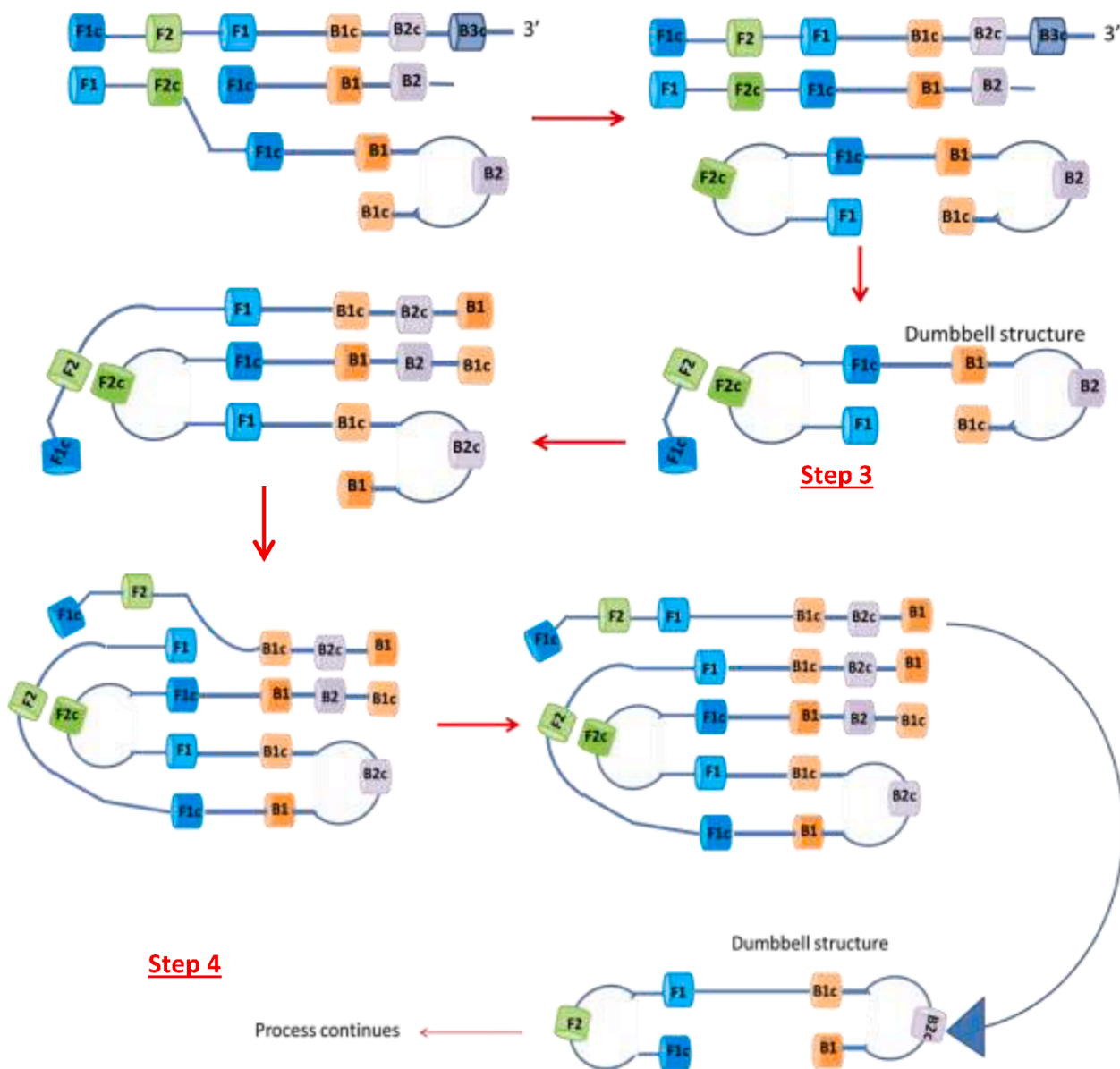
Fig. 3. (*continued*).

Immuno-assay or DNA-based assays have been the most commonly used techniques to quantify and identify the pathogens. However, the selection of assay to be used depends on various factors such as the availability of antibodies, stage of an infection and DNA sequence data, which include viral DNA, species- and strain-selective genes and toxin-producing genes. Detection of antibodies in the infected organism, either during or after infection, may offer detailed information about the infecting pathogen. The characteristics in such assays are that the antibodies are the bio-recognition element as well as the targeted moiety. Immunoassays can therefore be used to detect directly the pathogen using the available antibodies. The advantageous property of immuno-assays to be used in the direct and indirect detection of pathogens via the generated antibodies and pathogen epitopes, make these techniques a flexible approach to detect the pathogens. On other hand, if the availability of antigens is limited or antigen production in the organism is significantly lower even in the presence of pathogen causing infection, then DNA-based assays can be commonly employed though these techniques require the sample to contain pathogen at the time of analysis.

The well-known bio-analytical techniques usually detect one or more components in the sample using a molecular probe as a bio-recognition element combined with the analytical system such as PCR analyzer or a plate reader. However, the robustness and sensitivity of these techniques are advantageous, and these techniques have offered time-to-results due to extensive reagent utilization in sample and complex sample preparation steps. In addition, as discussed in the previous section, PCR-based bio-analytical methods may also be affected due to contextual species in the sample, resulting in a bias and uncertainty of the measurements [53]. Considering the various limitations of traditional methods and continued requirements, real-time analysis is always a better alternative.

PCR and ELISA based techniques for the detection of pathogens have complimented the biosensors for over 25 years, wherein a selective transducer is integrated with a bio-recognition element providing a platform for the identification and quantification of the infecting pathogen. As per International Union of Pure and Applied Chemistry (IUPAC), biosensor is categorised with characteristics such as direct contact between bio-reorganisation element and transducer element to provide semi-qualitative or qualitative analytical information excluding the reagents and additional processing steps. Hence, it is a self-contained

integrated device [54]. Significant and advantageous developments in biosensors have led to the fabrication of devices enabling to selective real-time detection of pathogens in various environments and matrices such as surfaces, body fluids foods, and wastewaters.

The fabrication of biosensor that can detect biological analyte purely based on their intrinsic properties, would be a difficult task. Hence, in addition to protocols that are free from sample preparation, labelled and label free protocols for the fabrication of biosensors have been proposed [55]. Labels such as enzymes and fluorescent or radioactive molecules are attached to the targeted analyte [56] and the sensor signal corresponds to the amount of labels, representing the number of bound target molecules. However, labelled protocols have some adverse drawbacks such as they are cost-intensive, sophisticated preparation facilities, trained personnel, time-consuming and they can block the active binding sites, resulting in an alteration of binding properties, thereby affecting the affinity-based interaction between the recognition elements and the target molecules [57]. Table 1 gives a summary of the utilization of some of the methods to detect different viruses, but in any case, as per literature the PCR-based analysis of viruses is the first choice Fig. 4 describes Table 1, for number of articles reviewed based on different viruses, methods and the year.

4.1. Advances in electrochemical sensors for the detection of viruses

Lowering the detection limit is the key for early detection of the infection and individuals are not infectious before they are normal. In such situations, electrochemical methods play a vital role. Further, this is easy to fabricate miniature devices to be useful at the point-to-care, offering immediate and reliable results. However, construction of electrochemically-based biosensors depends on the components such as transducer element, bio-recognition elements, and measurement formats.

In case of an electrochemical biosensor, transducer element is a cell consisting of three electrode system (potentiostat) or a two electrode system (conductometry and electrochemical impedance spectroscopy) in which much importance relies on the working electrode [58-60]. The working electrode can be fabricated with semiconducting and conducting materials ranging from metals to non-metals such as carbon, and using the materials of various sizes from bulk materials to micro and nano-structures. The electrode properties and structures affecting the performance of the electrode in terms of selectivity and limit of detection are dependent on the materials used, fabrication methods employed and the design approach.

Various metal-based electrodes consisting of gold and platinum are used as biosensors [107-109]. For instance, thick metal surface or a thin film metal electrode have been fabricated by cutting or traditional micro-fabrication using physical vapour deposition and screen printing techniques [110,111]. In addition, ceramic electrode (consisting of polysilicon, TiO_2, and indium tin oxide) and polymer electrodes (with advantageous properties of stability, biocompatibility and tuneable electric conductivity) have also been used in the fabrication of electrodes [112,113]. However, the selection of materials for the fabrication of electrochemical sensors, especially while detecting pathogens requires expert skills. Since significant aspects of electrochemical sensor performance such as rate of heterogeneous electron transfer, double layer capacitance, nature of coupling chemistry required immobilising the bio-receptors may be affected. Furthermore, since Faradic current is dependent on the active electrode surface area, increasing the surface area improves the sensitivity as well as controls the background current. A simple and effective mode to increase surface area is to use the nanomaterial and the composites. This would also facilitate easy immobilization of bio-receptors, thus increasing the sensitivity in a wider dynamic range, thereby allowing higher collision frequency between the antigens and the antibodies [114].

Elevating the target and/or selective binding based on enzymes or antibodies are the principal needs for biomolecular recognition. Limited

Table 1
Recently used methods for viral detection.

Parasite	Method	Limit of Detection	Ref.
a) Measles virus (MeV) b) Rubella virus (RV) c) Human enterovirus (EV) d) Varicella-zoster virus (VZV) e) Dengue virus (DENV) f) Human parvovirus B19 (B19) g) Epstein-Barr virus (EBV) h) Human herpes virus 6	Multiplex real-time RT-PCR	a) MN (copies/reaction) = 104 MX(copies/reaction) = 94 b) MN (copies/reaction) = 301 MX (copies/reaction) = 81 c) MN (copies/reaction) = 190 MX (copies/reaction) = 137 d) MN (copies/reaction) = 68 MX (copies/reaction) = 70 e) MN (copies/reaction) = 203 MX (copies/reaction) = 177 f) MN (copies/reaction) = 58 MX (copies/reaction) = 60 g) MN (copies/reaction) = 49 MX (copies/reaction) = 47 h) MN (copies/reaction) = 43 MX (copies/reaction) = 72 MN: Monoplex; MX: Multiplex	[61]
Gastroenteritis virus VN-96	Electro-optical sensor	~104 viral particles ml^{-1}	[62]
African swine fever virus (ASFV)	Polymerase chain reaction with a lateral flow strip	1.5×10^1 copies/reaction	[63]
Japanese encephalitis virus (JEV)	Fluorescence molecularly imprinted sensor based on a metal–organic framework	13 pmol L^{-1}	[64]
a) Tentative names: Ginger chlorotic fleck associated virus 1 (GCFaV-1) b) GCFaV-1 c) GCFaV-1 d) GCFaV-1 e) Tentative names: Ginger chlorotic fleck-associated virus 2 (GCFaV-2). f) GCFaV-2 g) GCFaV-2 h) GCFaV-2	a) RT-LAMP b) RT-RPA c) RT-PCR d) Real-time RT-PCR e) RT-LAMP f) RT-RPA g) RTPCR h) Real-time RT-PCR	a) 10^{-5} b) 10^{-4} c) 10^{-7} d) 10^{-2} e) 10^{-3} f) 10^{-3} g) 100 h) 10^{-5}	[65]
a) Duck hepatitis A virus 1 (DHAV-1) b) DHAV-3 c) Duck astrovirus 1 (DAstV-1) d) DAstV-2 e) Duck reovirus 1 (DRV-1) f) DRV-2	Matrix assisted laser desorption / ionization time of flight mass spectrometry	a) 4.0 copies b) 7.3 copies c) 1.3 copies d) 2.1 copies e) 1.3 copies f) 3.3 copies g) 3.9 copies h) 1.7 copies i) 3.4 copies j) 7.8 copies	[66]

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Table 1 (*continued*)

Parasite	Method	Limit of Detection	Ref.
g) Tembusu virus (TMUV)			
h) Avian influenza virus (AIV)			
i) Goose parvovirus (GPV)			
j) Duck enteritis virus (DEV)			
Dengue virus	Reverse transcription recombinase-aided amplification with lateral-flow dipstick assay	10 copies/mL	[67]
Porcine epidemic diarrhoea virus	SYBRTM Green one-step RT-qPCR	50 genome copies/5μl of extract from fecal matrices spiked100 genome copies/5μl of extract from jejunum matrices spiked	[68]
Herpes simplex and varicella-Zoster virus	Real-time polymerase chain reaction (RT-PCR) (Argene, BioMerieux, France) performed on an LC480 platform and isothermal amplification using a Solana HSV1 + 2/VZV assay	107 copies/mL for HSV-1, HSV-2, and VZV	[69]
African Swine Fever Virus	CRISPR-Cas12a and fluorescence based point-of-care system	1 pM	[70]
Laryngotracheitis virus	Colloidal gold test strip based on membrane chromatography	60 ELD 50/ mL	[71]
Dengue and Zika viruses	Multiplex RT-qPCR assays	duplex assay was 0.028 and 0.065 FFU (focus forming unit)/ml for DENV and ZIKV respectively	[72]
Nervous necrosis virus	A lateral flow immuno-chromatic strip	$10^{5.05}$ TCID50/ 100 μl	[73]
Hepatitis B virus	Electrochemical DNA sensor based on nanoflowers of Cu3 (PO4)2-BSA-GO	1100 copies/mL for HBV-DNA	[74]
Japanese Encephalitis Virus	Duplex TaqMan RT-qPCR	10 genomic copy	[75]
a) Porcine epidemic diarrhea virus	Duplex real-time PCR assay based on SYBR Green I	a) 10 copies/μL b) 10 copies/μL	[76]
b) Porcine bocavirus (PBoV) 3/4/5			
Zika virus	a) Colorimetric format. b) Colorimetric format. c) Electrochemical format d) Electrochemical format	a) Genosensors R1: 32 pmol L-1 b) Genosensors R2: 9 pmol L-1 c) Genosensors R1: 0.7 pmol L-1 d) Genosensors R2: 3 pmol L-1	[77]
a) Zika virus	Multiplex RT-qPCR	a) 100 copies b) 5 copies c) 50 copies	[78]
b) Chikungunya viruses -a			
c) Chikungunya viruses -b			
Porcine epidemic diarrhea virus	Immuno-chromatographic strip	1:50	[79]
a) H1N1 of influenza A virus	CdSe/CdS/ZnS quantum dot-linked rapid fluorescent immunochromatographic test	a) 28.37 b) 34.48	[80]
b) H3N2 of influenza A virus			
Epstein-Barr virus	Electrochemical detection	0.46 fM	[81] [82]

Table 1 (*continued*)

Parasite	Method	Limit of Detection	Ref.
a) Pseudorabies virus	SYBR green I-based duplex real-time PCR	a) 37.8 copies/μL, b) 30.6 copies/μL	
b) Porcine circovirus 3			
a) Classic swine fever virus	SYBR green I-based duplex real-time fluorescence quantitative PCR	a) 23 copies/μL b) 36 copies/μL	[83]
b) Porcine circovirus 3			
Ebola virus	Rolling circle amplification of Ebola virus and fluorescence detection based on graphene oxide	1.4 pM.	[84]
Ebola virus	Microfluidic sample preparation multiplex	0.021 pfu/mL	[85]
Ebola virus	Electrochemical DNA biosensor	4.7 nm	[86]
Ebola virus	RT-PCR	10 molecules/μl	[87]
Ebola virus	Fluorescently-labeled phosphorodiamidate probe pairs	25 pM	[88]
Ebola virus	Real-time reverse transcription-polymerase chain reaction	10 copies per reaction	[89]
Ebola virus	Real-time reverse-transcription PCR assay	5×10^2 viral particles per ml	[90]
Ebola virus	Reverse-transcription-PCR (RT- qPCR)	0.374 cps/μl	[91]
HIV	Ultra-sensitive electrolyte-gated field-effect transistor	HIV-1 p24 proteins at a concentration of 1 fM,	[92]
HIV	Molecularly imprinted electrochemiluminescence	0.3 fM	[93]
HIV	DNA-stabilized silver nanoclusters (AgNCs)-based label-free fluorescent platform	11 pM	[94]
HIV	Electrochemical DNA sensor, polyaniline/graphene nanocomposite	1.0×10^{-16} M	[95]
HIV	Wearable microfluidic device combined with recombinase polymerase amplification	100 copies/mL	[96]
HIV	Impedimetric	2.5×10^{-12} molL^{-1}	[97]
HIV	Luciferase immunosorbent assay	10 pg/mL and 100 ng/mL was reached for LISA and ELISA	[98]
Hantavirus	Electrochemical immunoassay	LOD of 0.14 ng mL − 1	[99]
Dengue	Immunofluorescence Colorimetric	15 ng mL^{-1}	[100] [101]
Avian influenza (AIV H5N1)	Surface plasmon resonance	1 pM	[102]
a) classical swine fever virus	Multiplex RT-PCR	1×10^3 copies	[103]
b) porcine epidemic diarrhea virus			
c) porcine reproductive and respiratory syndrome virus			
d) transmissible gastroenteritis coronavirus (TGEV)			
Influenza A (H1N1)	RT-LAMR mediated colorimetric determination	3×10^{-4}	[104]
Influenza a (pH1N1)	Polydiacetylene (PDA)-based colorimetric biosensor	10^5 TCID$_{50}$ without reading device10^4 TCID$_{50}$ with reading deviceThrough colour change	[105]

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Table 1 (*continued*)

Parasite	Method	Limit of Detection	Ref.
Zika Virus	qRT-PCR	from $5 \times 10^3 \sim 10^4$ TCID$_{50}$ viruses 2.5 PFU/mL	[106]

stability of these complex materials are often accompanied with multi-faceted protocols and specific handling protocols. For virus detection receptors to be reused they can mimic antibodies recognition properties that are favourable, especially in health care systems. Hence, recent efforts on molecular imprinting strategies have evolved significantly allowing the fabricated sensor to mimic immunological interactions [115].

In molecular imprinting, the first step involves the interaction between cross-linking agents and the monomers in a suitable solvent with the templates; then following the arrangement of formed molecular assemblies by PCR around the template molecules, and finally removing the templates leaving behind the analyte selective binding moieties. Recently, extensive studies based on molecularly imprinted polymers have detected a wide range of species targeting proteins, cells and viruses [116,117]. These included different polymerization strategies such as surface imprinting (2D) and bulk imprinting (3D). To perform the bulk imprinting, the respective template was directly added to a monomer mixture and the hydrogels formed with 3D matrices could offer less restricted diffusion pathways [118,119].

Diverse examples of hydrogels used to imprint viruses are available in the literature [120-122]. On other hand, surface imprinting can be achieved by attaching the template to the supporting material or by a thin polymer film decoration. These methods can be carried out using soft lithography, self-assembly or by core–shell particles via immobilized templates. On the other hand, the traditional imprinting techniques have focussed on materials that can favour small molecular templates. Key issues such as solubility, size, fragility, and compositional complexity are to be considered while imprinting the viruses.

The fabrication of electrodes using carbon materials has advanced significantly and classical carbon-based sensors are mainly glassy carbon, carbon fibers and pyrolytic graphite (Fig. 5) [123,124]. Most of the carbon-based nanomaterials have many advantageous properties such as higher electro-catalytic, adsorption bio-compatibility and fast electron transfer rate [125]. For sensor applications, carbon nanotubes and graphene have been investigated as these can be directly incorporated into a biological sensor following the simple drop casting, growing the material directly on the substrate, co-depositing with metal nanoparticles and then using them in field effect transistors [126].

Wasik et al. [127] developed an electronic biosensor based on heparin functionalized carbon nanotubes for the detection of dengue virus. Among the heparin, heparin sulfate proteoglycans have been used as receptors for dengue virus during the infection of Vero cells to detect the lowest concentration up to 8.4×10^2 TCID$_{50}$/mL ~ 8 dengue virus/chip. This method was chemiresistor functionalized with heparin instead of using an antibody for the detection of dengue virus (Fig. 6).

The single walled carbon nanotube network can be synthesised by self-assembly on gold electrode lithographically where the primary amine linker, 1 –pyrenemerhylamine can be adsorbed on the single walled carbon nanotubes that are cross-linked with heparin carboxyl groups and used for detecting the dengue virus. Selectivity of the biosensor was evaluated [127] using influenza virus H1N1 as the negative control.

Navakul et al. [128] demonstrated electrochemical biosensor based on impedance spectroscopy using gold electrode deposited with graphene oxide to detect in the limit of 0.12 pfu/ml. Schematic representation of the deposition of graphene oxide polymer onto the surface of gold electrode is shown Fig. 7. The specificity of fabricated electrode was assessed since the copolymer was electrically conductive due to the presence of graphene oxide. Further, the negative charge of oxygen atom in graphene attracts the dengue virus particles with a positive potential. Joshi et al. [129] demonstrated a method using thermally reduced graphene oxide deposited onto indium tin oxide/glass electrodes for quantitative determination of influenza virus H1N1 as a label free electrochemical immuno-sensor using impedance spectroscopy. The detection limit reported by this method was 26 and 33 pfu/mL for saline and diluted saliva, respectively (Fig. 8).

Bhattacharya et al. [130] demonstrated layer-by layer assembled functionalized carbon nanotubes for interaction with antiviral antibodies and avian metapneumo virus. A form of resistor was developed patterning onto gold electrode on a Si/SiO2 substrate onto which layer-by-layer assembly of carbon nanotubes were built. The poly(diallyldimethylammonium chloride), poly(styrene sulfonate), and functionalized single-walled carbon nanotubes were absorbed onto the surface electrostatically to form the multilayer films. The interaction and immobilization of viral antibodies between the electrode was
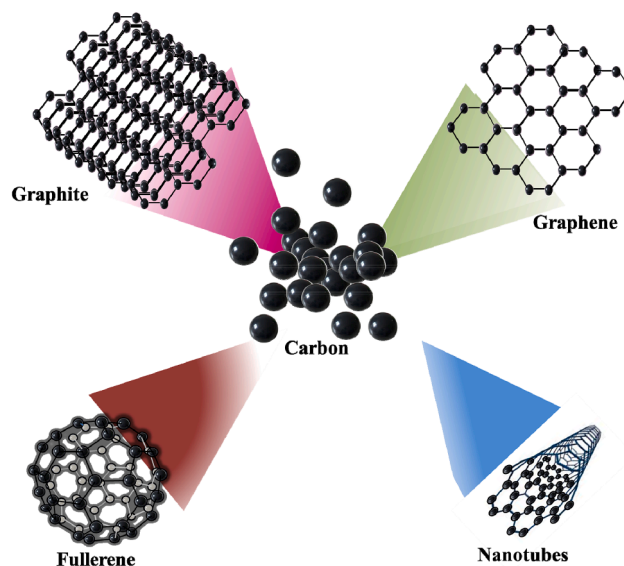
Fig. 5. Most commonly used carbon materials for sensor application.
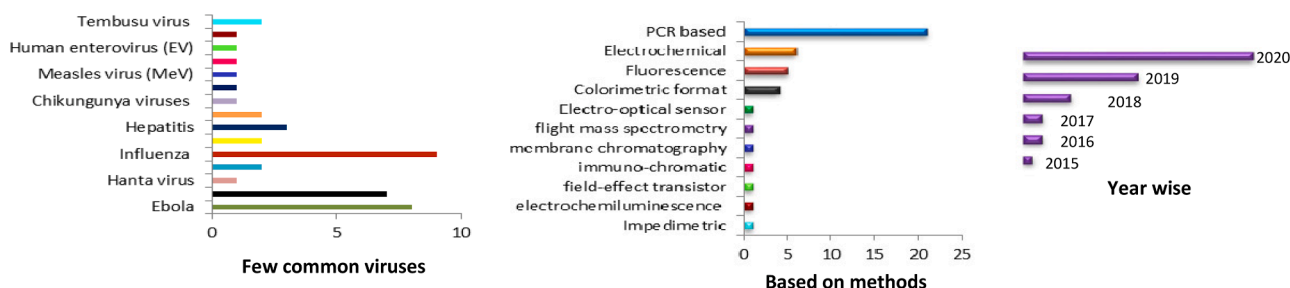
Fig. 4. Description of Table1, number of articles reviewed based on different viruses, methods and the year.
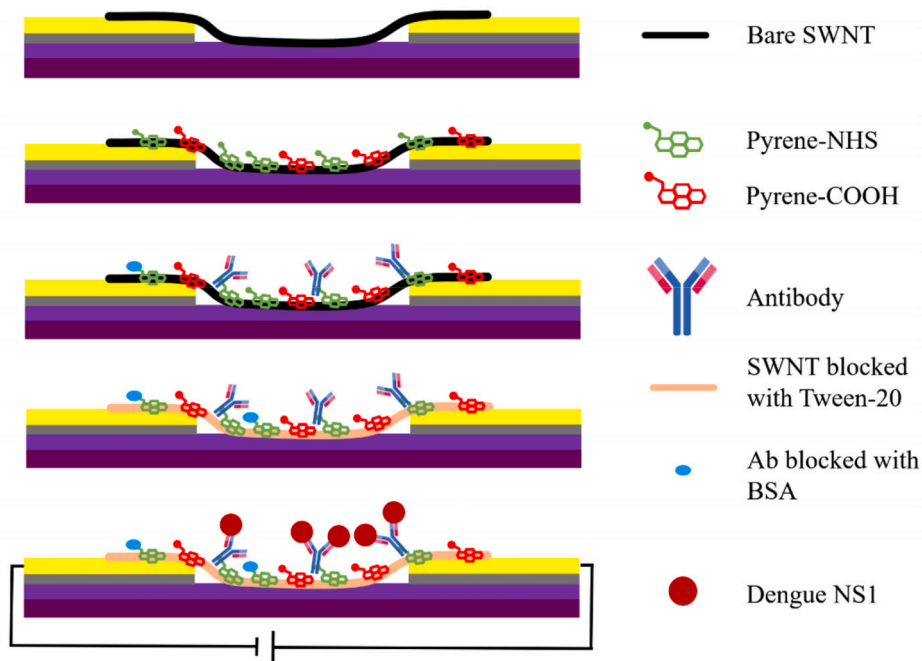
Fig. 6. A schematic representation of the fabrication of chemiresistor used for the detection of dengue virus (from Ref. 127).
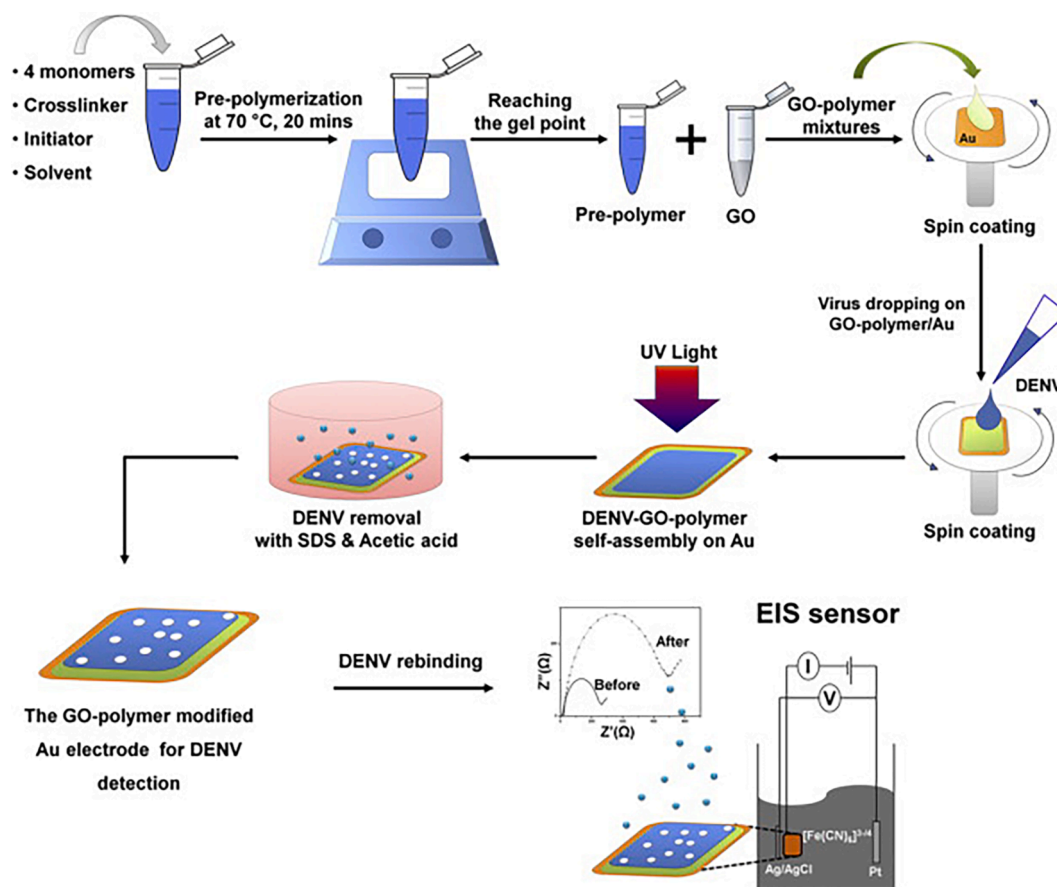
Fig. 7. Schematic representation of the preparation of GO-polymer on gold electrode for DENV detection (from Ref. 128).

enhanced using poly(L-lysine), further capturing the antibody specific antibodies. This device was able to detect the change in conductivity with an antigen of 10^2 TCID$_{50}$/mL.

Fu et al. [131] developed chemiresistive biosensor based on carbon

nanotubes for detecting avian influenza virus H5N1. In these sensors, long nanotubes (>5 _m) were placed between the inter-digitised metal electrodes such that individual nanotubes connect the electrodes and nanotubes were functionalized with DNA probe sequences non-
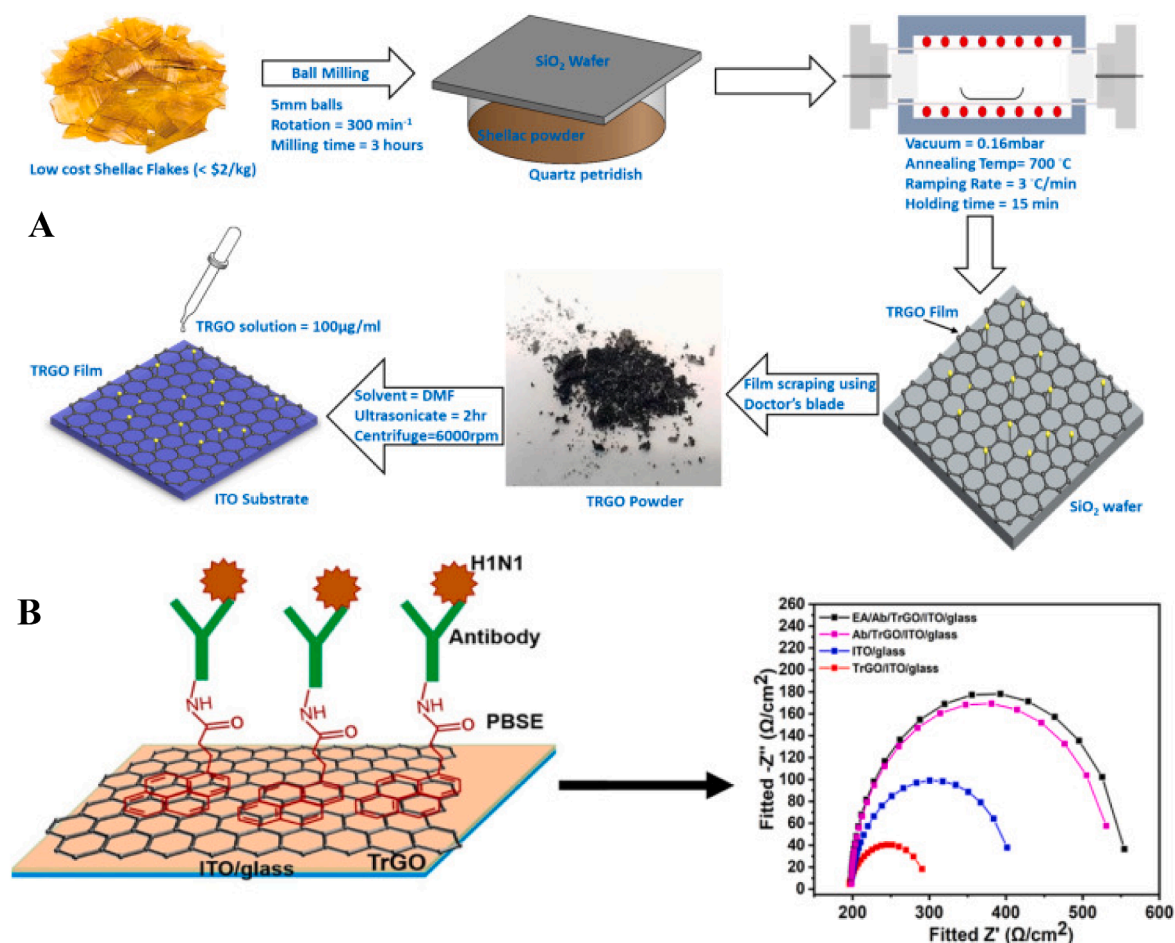
Fig. 8. (A) Schematic illustration to display the synthesis route of TrGO using Shellac biopolymer; (B) Schematics of the proposed thermally-decomposed reduced graphene oxide (from Ref. 129).

covalently attached to the sidewalls. These functionalized-nanotube sensors reliably detected the complementary DNA target sequences of AIV H5N1 within a concentration range between 2 pM and 2 nM in 15 min at room temperature. Table 2, represents various electrochemical methods for detection of pathogens proposed recently.

5. Application of nanotechnology

In recent years, outstanding achievements in nanotechnology have allowed novel materials to steadily intensify their new horizons across the globe (Fig. 9) [167-175].

Flexibility and expendability of the nanomaterials are prowling in biomedical areas as some have almost reached commercialization, especially for viral detection [176]. In addition, such advances have led nanotechnology with remarkable opportunities for the detection and diagnosis of viral infections since these materials have unique properties compared to their bulk materials and have significantly enlarged surface-to-volume ratio. Surface properties, size and composition of nanomaterials can be engineered to develop robust materials with superior luminescent and electrochemical properties.

Metals and metal oxide nanoparticles in the size range of 1–100 nm are significantly suitable to fabricate biosensors due to their noticeable increase in surface area to volume ratio since the size of the material decreases. For instance, in biomedical area, unique optical and electric properties of gold nanoparticles have been well established for the detection of viruses. Shariati et al. [177] proposed label free detection of human papilloma virus based on gold nanotubes using the electrochemical impedimetric technique. In this study, external electric field

applied allowed the preferred orientation of the negatively charged DNA oligonucleotide to increase the sensing response via controlled hybridization and immobilization of the sequence onto gold nanotubes surface. This biosensor has shown a lower detection limit of 1 fM in the linear range of 0.01 pM to 1 μM.

Lee et al. [178] developed an electrochemically based label-free avian influenza virus detection method using multi-functional DNA structure on a porous (p) AuNPs-modified electrode. The proposed DNA 3 way-junction/pAuNPs based detection method can be applied for multiple-target detections as a valuable biosensor for determining the pathogen subtype in one platform or one target detection using the dual detection method with high reliability (Fig. 10).

Zhao et al. [179] demonstrated a stable electrochemical method for the detection of hepatitis B virus based on nanoflowers of $Cu_3(PO4)2-$, gold nanoparticles by amplifying the signals using two aptamers. The fabricated sensor showed excellent binding points along with adaptive outline for the amplification of signal and biocompatibility. The use of 3D nanoflowers was advantageous to increase the surface area, reaction kinetics, carrier immobility, and charge transfer. The structural features such as petals and their dimensions were controlled by synthesizing a new organic and inorganic framework based on $CuSO4··5H2O$, bovine serum albumin and graphite oxide. The extra binding sites were further obtained using gold nanoparticles to enhance the interaction between electrode and thiol-functionalized Aptamer-1. The detection limit was 1100 copies/ml for hepatitis B virus DNA in a dynamic linear range of 1.10×10^3 to 1.21×10^5 copies/mL.

Silver nanoparticles offer advantages of simple preparation and have good binding ability with biomolecules that can offer excellent

Table 2
Electrochemical methods used for the detection of pathogens.

Electrochemical methods	Type of electrodes	Targeted virus	Limit of detection	Ref
Conductometry	Ag nanofiber array electrode	Bovine viral diarrhoea virus (BVDV)	103 CCID/mL	[132]
Cyclic voltammetry	Graphene microelectrode	Rotavirus	$10\ ^3$PFU/mL	[133]
EIS	Au electrode	Human influenza A virus H3N2	8 ng/mL	[134]
Conductometry	PDDA/CNT composite on Au microelectrode	Swine influenza virus (SIV) H1N1	180 TCID50/mL	[135]
DPV	Nanostructured alumina on Pt wire electrode	Dengue type 2 virus (DENV-2)	1 PFU/mL	[136]
EIS Ferrocene methanol	Nanostructured alumina on Pt wire electrode	DENV-2	1 PFU/mL	[137]
Conductometry	Silicon nanowire electrode array	Human influenza A viruses H1N1 and H3N2	2.9×10^4 viruses/mL	[138]
EIS; Fe(CN)63-/4-	Au microelectrode	Human influenza A virus H1N1	–	[139]
EIS; Fe(CN)63-/4-	Pt-coated nanostructured alumina membrane electrode	DENV-2, dengue virus 3 (DENV-3)	0.23 PFU/mL	[140]
Amperometry	Polypyrrole nanoribbons on Au microelectrode array	Cucumber mosaic virus (CMV)	10 ng/mL	[141]
SSWV, fluorescence	AuNPs on carbon electrode	Murine norovirus (MNV)	180 viruses	[142]
Amperometry	Reduced graphene oxide	Rotavirus	100 PFU	[143]
CV, EIS; Fe(CN)6	AuNPs on Au electrode	Dengue virus 1–4		[144]
EIS; Fe(CN)$^{3-/4-}$	Au interdigitated microelectrode array	Avian influenza virus (AIV) H5N1	4 HAU/mL	[145]
SWV	Au microelectrode	Norovirus	10 PFU/mL	[146]
EIS	Au interdigitated microelectrode array	Avian influenza virus (AIV) H5N1	4.2 HAU/mL	[147]
EIS	Au electrode	Human influenza A virus H3N2	1.3×10^4 viruses/mL	[148]
DPV	Graphene/AuNP composite on carbon electrode	Norovirus	100 pM	[149]
CV, EIS	Au electrode	Norovirus	7.8 copies/mL	[150]
CV, EIS	Carbon NPs on carbon electrode	Japanese ncephalitis virus (JEV)	2 ng/mL	[151]
EIS, potentiometry	PEDOT film electrode	Human influenza A virus H1N1	0.013 HAU	[152]
Chrono-amperometry	Reduced graphene oxide on Au	Human influenza A virus H1N1	0.5 PFU/mL	[153]
Amperometry	PEDOT:PSS film electrode	Human influenza A virus H1N1	0.015 HAU	[154]
EIS;	Au electrode	Norovirus	1.7 copies/mL	[155]
EIS		Avian influenza	0.26 HAU/mL	[156]

Table 2 (*continued*)

Electrochemical methods	Type of electrodes	Targeted virus	Limit of detection	Ref
	Au interdigitated microelectrode array	virus (AIV) H5N1		
EIS	Au interdigitated microelectrode array	Avian influenza virus (AIV) H5N1	10^3 EDI50/mL	[157]
EIS	Au interdigitated electrode array	Avian influenza virus (AIV) H5N1	0.04 HAU/mL	[158]
ASV	AuNPs on ITO microelectrode	Avian influenza virus (AIV) H5N1	10 pg/mL	[159]
CV	Au electrode	Avian influenza virus (AIV) H5N1	0.367 HAU/mL	[160]
Chrono-amperometry	Carbon electrode	Human influenza A virus H9N2	16 HAU	[161]
CV, EIS	AuNPs on ITO electrode	Human enterovirus 71 (EV71)	10 pg/mL	[162]
SWV	AuNPs on carbon electrode	Middle East respiratory syndrome	400 fg/mL	[163]
DPV	GCE	Inflenza virus A	0.43 pg/mL	[164]
LSV	GCE	H7N9 virus	6.8 pg/mL	[165]
EIS	Gold electrode	Avian Influenza virus	8 ng/mL	[134]
Chrono-amperometric, DPV	Carbon SPE	H5N1 and H1N1 virus protein	8.3 pM (H5N 1) and 9.4 pM (H1N1)	[166]

opportunities for the fabrication of electrochemical biosensors. Khristunova et al. [74] developed an electrochemical immuno-sensing technique for the detection of antibodies to tick-borne encephalitis (TBEV). Thiolation and glutarization of the composite electrode containing gold-carbon was performed prior to covalent immobilization of the antigen onto the surface of the electrode. Further, measurement of released silver species from the silver nanoparticles containing bio-conjugates with antibodies to TBEV was the basis of assay of this method. The proposed method was suitable to quantify antibodies to TBEV in the range of 100–1600 IU mL^{-1}, with a LOD of 90 IU mL$^{-1.}$

Nanomaterials based on graphene are advantageous due to their outstanding chemical, mechanical, thermal and electronic properties and these are quite predominant to design the biosensors for DNA detection due to their enhanced affinity towards single-stranded DNA by the hydrophobic interaction and π-π stacking [180]. Graphene can be used as a substrate to interface with different cells and biomolecules, which is beneficial to improve its biocompatibility, solubility and selectivity

Li et al. [181] proposed DNA-assisted magnetic reduced graphene oxide-copper nanocomposite for the sensitive detection of hepatitis C virus DNA in the linear range of 0.05 to 10 nm with a detection limit 405 pM. The copper ions assisted for accelerated oxidation of o-phenyl-enediamine producing 2, 3-diaminobenazine from which electrochemical signals were obtained for the characterisation of hepatitis C virus DNA. Wang et al. [182] developed a sandwich type of electrochemical immunoassay for the sensitive detection of avian leukosis virus subgroup J using graphene quantum dots and apoferritin- encapsulated Cu nanoparticles. Differential pulse voltammetry was used to detect the released Cu from apoferritin cavity from the assembly. The electric
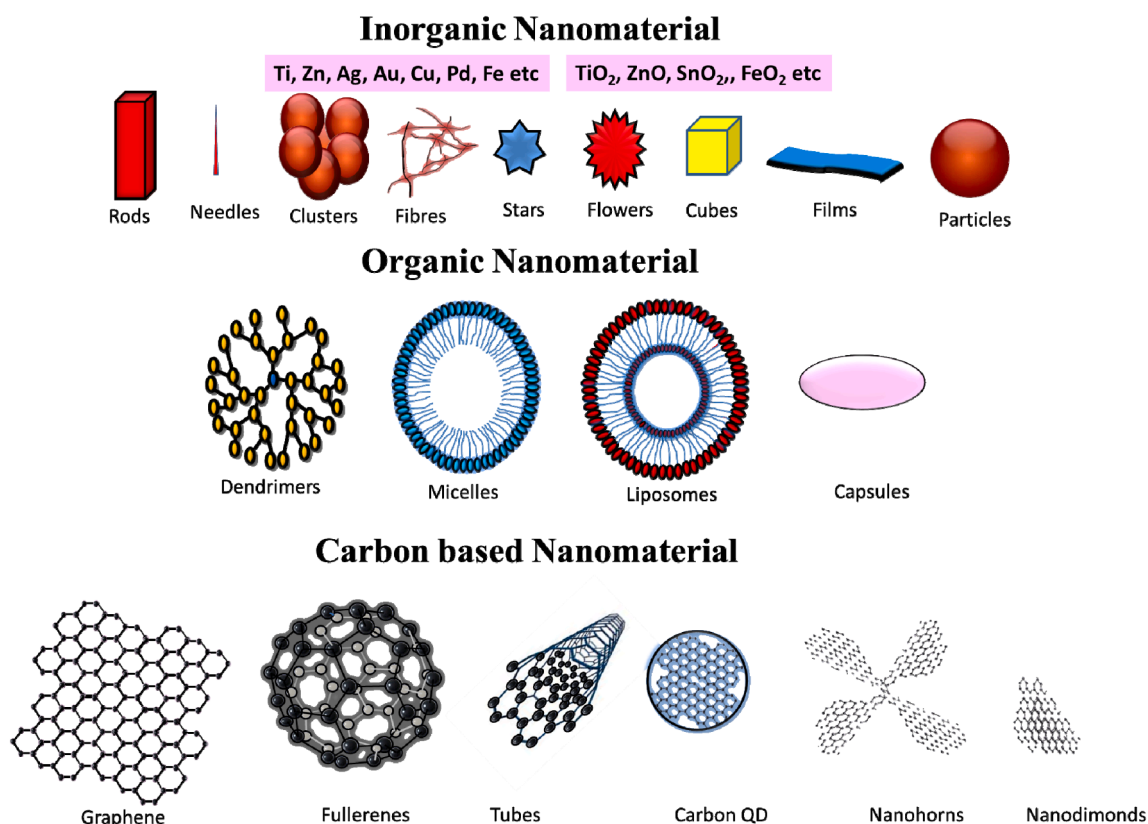
Inorganic Nanomaterial

Ti, Zn, Ag, Au, Cu, Pd, Fe etc   TiO₂, ZnO, SnO₂,, FeO₂ etc

Rods   Needles   Clusters   Fibres   Stars   Flowers   Cubes   Films   Particles

Organic Nanomaterial

Dendrimers   Micelles   Liposomes   Capsules

Carbon based Nanomaterial

Graphene   Fullerenes   Tubes   Carbon QD   Nanohorns   Nanodimonds

Fig. 9. Nano based material for sensor application.

HA Protein
(AIV Coat Protein

Porous AuNP
Hybrid Nanostruc

Multi-Functional
(AIV detection, E
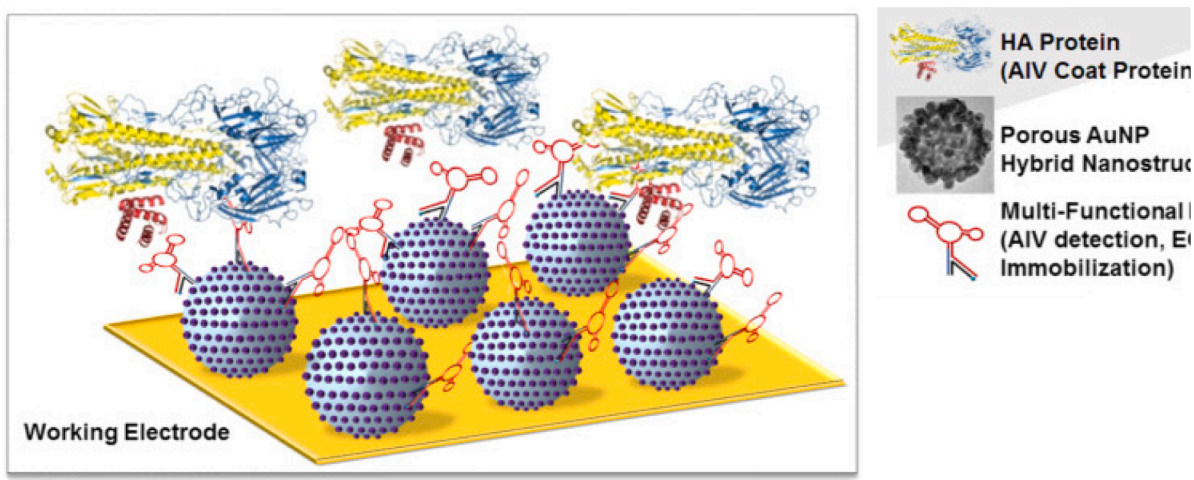Immobilization)

Working Electrode

Fig. 10. Schematic image of the fabricated AIV detection biosensor. (From Ref. 179).

signals increased effectively owing to the huge surface area of graphene quantum dots accommodating a considerable amount of antibodies loading. Here, Cu-apoferritin nanoparticles were responsible to increase the loading of electroactive probe to further amplify the signals. The method could detect avian leucosis virus in the range of $10^{2.08}$ to $10^{4.50}$ $TCID_{50}/mL$ with a detection limit of 115 $TCID_{50}/mL$.

Acute and chronic diseases caused by various viruses pose real threat to the human. These include smallpox, influenza, diarrhea, AIDs, hepatitis and polio, which have devastating effects that need initial cautionary system for the recognition and detection of the virus. Use of nanotechnology in the initial recognition of viruses is interesting and eye catching. Literature reports suggest the application of nano-

biosensors, immobilization schemes and immuno-sensing for the detection of various viruses. The advantageous applications of nanomaterials in addition to electrochemical nano-biosensors have been useful for the development of several diagnostic procedures in medical arena. Their applications are wide-spread in the quantification of numerous clinical biomarkers, evaluation as well as follow up after the illness. At present, graphene, magnetic nanoparticles, carbon, gold silica, and quantum dots have provided a subtle and precise strategy in biosensing.

The highly promising ability of a biosensor using electrochemical nanosensor has to address many challenges, one of which is to establish itself at the point-of-care. In addition, the profit guarantee in bio-sensing

relies largely on the selection of an appropriate nanomaterial to function as a useful biosensor. Further, the risk of errors and mistakes in the detection of virus are critical when considering the immobilization method of concerned nanomaterial. Reusable and portability are the other two factors with properties such as easy discrimination of viruses along with sensitivity and selectivity level, which indeed need intense efforts in addition to lifetime of the assay. In summary, nanotechnology with much greater advances in the near future will certainly be a breakthrough in biomedical area to check and stop the viral diseases to provide healthy life.

In recent years, personalized medicine and digital health monitoring is becoming increasingly attractive and this tremendous potential has now become realistic due to the fabulous advances in skin interfaced wearable sensors. These sensors interface with the skin in a wide range of sizes from cellular level down to molecular level and these hold the capability for therapeutic and diagnostic functions with excellent precision, continuity and expediency. In addition, the new opening of adding artificial intelligence and integrated cloud-based technologies would enhance the utilization of smarter healthcare systems. These devices compared to the traditional healthcare systems, can collect non-invasive data from the human body to provide an insight into both fitness monitoring and medical diagnostics along with keeping a track of molecular biomarkers of the human system. For such sensor devices, electrochemical detection would probably be one of the most fitting techniques due to its easiness in miniaturization, and low electric power consumption. This review will not dare to dwell into these details as it is beyond the scope of the subject though some recent publications address these issues [183-191].

6. Rising test strategies for COVID-19 diagnosis

WHO in such current pandemic situation has given a suggestion for the development of techniques, which are rapid in response, especially that are based on nucleic acid and protein test formats [192]. These developed techniques should offer advantages for use in short-term at the point-of–care. Effectiveness for tracking and surveillance can be enhanced using serological test for the detection of protein. Further, emphasis on cost effectiveness, lowering the burden on clinical and central laboratories by easing the operation must be performed [193].

SHERLOCK method for the detection is one of the nucleic acid-based methods that has emerged and has all the potentials to be applied for the detection of SARS-CoV-2. Based on CSISPR method, a gene-editing tool used for RNA sensing using variants of Cas9, known as Cas13a ribonuclease. The process works by targeting the virus RNA followed by reverse transcription to DNA and isothermal amplification. Further DNA is transformed back to RNA where it interacts with Cas 13a. The targeted molecule activates Cas13a allowing the cleavage with fluorescent probe that yields the signal. Hou et al. [194] developed an isothermal, CRISPR-based method for the detection of SARS-CoV-2.

Detection of viral spike proteins and antibodies generated in the patients after the infection is another approach for the diagnosis of SARS-CoV-2. In detecting the coronavirus, antibodies studies have shown that S proteins from SARS-CoV-2 have greater reactivity. On other hand, an enzyme linked immuno-sorbent assay (ELISA) for detecting immuno-globins G and M in serum of the infected persons was demonstrated [195]. The studies used for nucleocapsid protein Rp3 from SARS-CoV-2 that has 90% similarity with SARS viruses. The results recorded on day 0 showed 50% positive for IgM and 80% for IgG, further increasing to 80% and 100% on day 5 [196]. The method has flexibility of sample such as blood, fecal and respiratory organs.

Considering the on-site testing that plays an important role in point-of-care that provides many advantage in which the key point is the on-spot detection avoiding the transport of samples to laboratories. One such a method proposed is the lateral flow assay, which is still under development for SARS-CoV-2. This method utilizes a paper strip coated with gold nanoparticles functionalized with antibodies. Simple colour change due to clustering through plasmon banding of gold nanoparticles are observed to derive the results. Such methods have been used for MERS-CoV, but challenges such as single usage and low sensitivity are yet to be addressed and further research is needed to be done on the amplification of readouts.

Another approach used is the designing of Microfluidic devices using a small chip consisting of micro-channels for the reaction. Such microfluidic device-based smart phone for detecting antibodies of sexually transmitted diseases has been demonstrated [197]. Though the sensitivity of this method is in the range of 90–100%, attempts to use this technology for SARS-CoV-2 are not still available and if achieved, it would be a viable option for detecting specific proteins and nucleic acid for SARS-CoV-2.

RT-PCR devices have faced major challenges such as requirement of skilled and trained staff, infrastructure, and it take at least 6–24 h for the results. Hence, these techniques are not efficiently used in conditions of rapid screening in crowded places where huge numbers of samples have to be tested. However, portable RT-PCR devices can be another option for SARS-CoV-2, since portable RT-PCR has been used in plant pathogenesis. These devices are portable and upon the addition of a target viral RNA, it can detect the host biomarkers. As the early investigations of SARS-CoV-2 have shown S and N proteins are predominant, nucleic acids of these can be targeted in these tests. The advantage of this device is that the analysis of RNA takes place in less than 2 h on site avoiding the need for sample transportation to laboratories [198].

Chemiluminesence immuno-assay is one such an approach that has been popularly used in the detection of infectious diseases. Recent studies have demonstrated the application of this method for SARS-CoV-2 [199-201]. Cai et al. [200] demonstrated a peptide-based magnetic chemiluminesence enzyme immuno-assay for detecting SARS-CoV-2 antibodies against ORF1a/b, N and S proteins. The positive rate for this method was 71.4% for IgG and 57.2% for IgM. In any case, the approach based on RT-PCR has the first line of defence in the diagnostic test of SARS-CoV-2. Efforts are being made to develop new serological tests since there is an urgent need for efficient on-site and multiplex platforms. Technologies are still in the developmental stage and more focus should be deployed on creating clear communication network.

7. Summary, challenges and recommendations

In recent years, serious public health issues and crises due to viral infectious diseases such as Ebola, SARS, MERS and SARS-CoV-2 have been experienced by the global community. Since, these viruses can easily spread in large populations rapidly; extremely accurate diagnostic methods that are streamlined and can be conducted in integrated fashion are much needed. Hence in this review, developing research activities related to the pathogen and integration of electrochemistry and nanotechnology has been suggested. Considering the SARS-CoV-2 RNA detection, literature suggests that more efforts are needed to overcome the problems related to amplification and sensitivity. The PCR-based techniques are one of the most promising and widely used techniques for the pathogen detection. In these techniques, LAMP has shown to be promising tool for SARS-CoV-2. Although false results persist, major advantages using LAMP include the speed of analysis. Compared to RT-PCR technique, the RT-LAMP methods are proven to be much faster. Thermal cycling in RT-PCR is another drawback compared to isothermal conditions used in LAMP in addition to high amplification efficiency, which is an add-on advantage for LAMP.

In recent years, miRNAs acting as the specific biomarkers have attracted in disease diagnosis, prognosis and prediction of therapeutic responses. However, short sequence and low expression level pose a challenging task for miRNA detection. Duplex-Specific Nuclease-Amplified Detection of miRNA showed much promising potential to improve the specificity and sensitivity for miRNA detection. These advantageous characteristics of Duplex-Specific Nuclease-Amplified techniques are attributed to impeccable discrimination between the

perfectly and non-perfectly matched duplexes. However, the technique has to undergo significant progress in standardization and application in clinical real sample detection, but the most unresolved problem is designing a sensing probe.

Nucleic acid sequence analyses have proven more effective in disease diagnosis and in clinical treatments than other conventional methods. Due to their flexibility, specificity and sensitivity, CRISPR/Cas methods have revolutionized the accessibility of robust diagnostic tool. Variety of diagnostic applications, such as imaging assays, biosensing assays, and target enrichment for the next-generation sequencing are performed using the variants of Cas9 that has enabled us to develop cost-effective and flexible tests. Further Cas proteins such as Cas12 and Cas13 along with collateral cleavage activities have eased the application of diagnostic tools for rapid and portable detection and carrying a great potential for point-of-care systems. However, some drawbacks associated with this method are: (1) due to inherent off-target effects, improved specificity is a concern in CRISPR-based detection; (2) addition of protospacer adjacent motif for CRISPR targeted recognition results in reduced range of sequences that can be detected due to sequence limitation; (3) the workload and possible cross-contamination between the samples are still a challenge when dealing with large-scale tests such as COVID-19 outbreaks.

Electrochemical sensors are now more advanced due to the exploitation of novel materials and advances in instrumentation technologies for sensitive and selective identification, detection and quantification of viruses. However, compared to other technologies with improved smartness and capability, electrochemical sensors continue to significantly contribute in health-care systems due to their advantages of simplicity in design, easiness of integration within devices, robustness, high analytical sensitivity, low power consumption and low cost.

Current scenario of the outbreaks in viruses causing respiratory diseases has shown the significance of rapid detection, control of samples to answer and the point-of-care devices that can be positioned in a community. Point-of-care industries have faced particular challenges to ease the health problems in case of COVID-19. The OECD countries have already reached massive scaling of testing (23 per 1000 population), predominantly using PCR and laboratory testing rather than the point-of-care devices. Lowering the number of samples is crucial in contact tracing, where centralised laboratories are continuing to centre core in containing the deadly pandemic. Further, in such pandemics, point-of-care industries may raise opportunities to include identification of recovered patients having antibody profile to address the re-infection. Significantly, these networks will include experts in technology, regulatory approval, commercialization, and manufacturing so as to accelerate the progress.

While being increasingly considered, issues such as ability to handle diverse properties of the real-world samples, shelf-life, cost of goods, adoption by the end users, the cost effectiveness and mass manufacturability aspects need to be considered early in the device development life cycle. Further, integration of wireless communication in a device to adapt artificial intelligence to acquire maximum information from the response allowing consultants to make a quick decision is necessary. Use of biocompatible materials would enable the fabrication of easy wearable as well as implanting devices, which are sustainable and nontoxic; in such uses, minimally invasive samples are to be considered.

On the whole, several proofs of concepts on the use of nano-biosensors in pathogen detection are available and the versatility of nano-sensors for several analytes is already demonstrated. The strength of nano-sensors suitability has been validated with real samples in clinical scenarios.

Integrating nanotechnology with electrochemical sensors is still a matter of debate as they are recently developed and are used only at the research level. Even though there is a slow progress, significant advances in smart phone technology have helped mobile health diagnostics to deploy in the developing countries. In the near future, electrochemical sensors embedded with nanomaterials will play a

crucial role in the pandemics.

As the nanomaterial-based biosensors have remarkable advantages in the diagnosis of numerous viral infections, these may be a strong contender in designing early diagnostic kit for SARS-CoV-2. Nanostructures based on graphene, gold and lanthanide-doped polysterene may have an advantageous platform in biosensor technology development. Along with nanomaterials, techniques such as impedemetric, amperometric, colorimetric have established their niche and are able to detect the virus within 10–100 min and hence, can provide a greater assistance in detecting the SARS-CoV-2 pandemic. The limited development in these techniques for SARS-CoV-2 is a major drawback, but in the near future, these may be an alternative for the PCR-based techniques. The biosensor-based techniques can deliver the requirement of low-cost, easy, rapid and real-time diagnosis by reducing the pressure on PCR-based techniques, which are costly and time-consuming.

The electrochemical biosensors rely on proteins such as antigen/antibody or nucleic acid viz., RNA/DNA and the yield may not be 100% due to contamination of these bio-receptors. In such CRISPR techniques, specific techniques that can depend on aerosol mediated diagnosis may produce new advantages of response time, selectivity and sensitivity as well as lack of sampler perturbation. In the lab-scales, better and lower limit of detection can be achieved due to easy modification of the electrode surface.

8. Conclusions

Recently, health and safety related issues due to the pathogenic infections have raised concerns regarding its identification and prevention. In such consequences, detection of pathogens has the key role, especially in food industries where inappropriate situations may arise due to the failure in detection of an infection. Researchers have been active in their efforts to develop rapid methods as the traditional methods require 7–8 days to yield results. A promising approach using biosensors may be the major avenues for achieving the target.

The present review provides a critical discussion on the presently used COVID-19 detection techniques along with recent developments in the areas of electrochemistry and nanotechnology for the detection of pathogens. Integration of these two techniques has produced fabulous results compared to other classical methods. Electrochemical biosensors have reached the limit of detection as low as colony-forming units and single plaque-forming units. The use of various nanostructured materials for electrode fabrication has been comprised of metal/metal oxides, carbon materials, polymers and composites. Even though literature suggests successful applications of the electrochemical sensors in pathogen detection, still a wide gap exists in such device availability at the point-of-care. In the near future, there may be still some chances of such pandemics to occur and hence, there may be a greater need of smart devices. Certainly, combining electrochemical sensors with nanotechnology would offer a ready avenue to solve the issues related to deadly pandemics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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