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Development of Biosensing Systems for the Detection of Corona Virus: A Mini Review

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ABSTRACT

Currently there are no effective anti-viral drugs for SARS-CoV-2, so the primary line of defense is to detect infected cases as soon as possible. The main goal of this review is to provide an overview on the newly developed methods and also potential techniques for the detection of viral diseases especially corona ones to help researchers around the world assess the advantages and disadvantages of methods and select the right one to efficiently control such diseases.

INTRODUCTION

On 30th January 2020 the WHO declared a global "public health emergency of international concern" (PHEIC) regarding the epidemic caused by the 2019 novel coronavirus (2019-nCoV), which started in a Wuhan seafood market, Hubei province [1]. The WHO has currently named the disease, which has spread so rapidly throughout the world, as coronavirus disease 2019 (COVID-19) [2].

The 2019 novel coronavirus (2019-nCoV) has also been renamed as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. Coronaviruses got their name from their morphology when observed under a microscope. Coronaviruses are zoonotic meaning that the viruses are transmitted between animals and humans [3]. Coronaviruses can be classified into four genera (α, β, γ, and δ), and these viruses are detected in a very wide selection of animal species, including humans [4]. Since the beginning of the twenty-first century, three kinds of coronaviruses have crossed the species barrier, causing deadly pneumonia in humans. These include the severe acute respiratory syndrome SARS-CoV [5], Middle-East respiratory syndrome (MERS-CoV) [6], and SARS-CoV-2 [7] coronaviruses.

VIRUS STRUCTURE AND FUNCTION

Viruses are small infectious agents and obligatory intracellular parasites that use the host cell's biosynthetic and metabolic machinery to survive and replicate their genomes, and in doing so, they cause damage and subsequent destruction of host cells, which ultimately leads to different types of diseases in humans.

Viruses infect all types of life form, and can use viral digestive enzymes to dissolve the host cell membrane, thereby gaining entry into the cell [8]. However, they do not contain various enzymatic systems apart from digestive enzymes which are used to enter the cell. So, because of their structure and their enzyme system as mentioned above, antibiotics are not effective for viral infections [9].

METHODS FOR VIRUS DETECTION

There are many techniques for the detection of viruses, the functions of which depend on virus type and virus particle properties. Herein, we review the most important types.

Immunofluorescence Methods

Immunofluorescence (IF) staining including the "indirect" fluorescent-antibody assay (IFA) are classified as fast methods for the detection of viral antigens with ideal specificity and sensitivity [10]. In other words, they are

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based on enzyme immunoassays (EIAs) techniques like the enzyme-linked immunosorbent assay (ELISA) and make use of similar protocols and materials, such as monoclonal antibodies.

Nucleic Acid Amplification Tests (NAATs)

Since the early twentieth century, the detection of viruses in clinical samples via molecular methods has become widespread. Diagnosis of viruses can be done directly using clinical samples and cell culture supernatants via specific nucleic acid probes, which can find their complementary target viral RNA or DNA sequences or by using NAATs [11]. While methods based on nucleic acids are functional for most viroids and some viruses, the technique that has been most successfully exploited is based on the Polymerase Chain Reaction (PCR).

Polymerase Chain Reaction (PCR)

In this technique, many copies of one DNA template are made using primers, enzymes, and variable temperatures [12]. This technique is quickly performed for diagnostic applications due to the generated fluorescent signal, which could be detected at a time ('real-time') or the end of the process ('endpoint') without the contamination risk. Real-time PCR methods have been established for three important targets. 1. Real-time PCR is quicker than ELISA to establish, considering the development of antibodies for new viruses, 2. This method is more functional, especially in routine laboratories, 3. Real-time PCR is more cost-effective due as compared to the expensive antibody production for immunosorbent assays.

Isothermal Amplification

The main theory of isothermal amplification of DNA is separating two strands of the template through nonthermal ways, such as helicase dependent amplification (HDA) [13] and Recombinase Polymerase Amplification (RPA) [14]. An alternative isothermal amplification approach is to design primers such that the extension products contain single-stranded primer binding sites [15]. Accordingly, loop-mediated isothermal amplification (LAMP) is the most ordinarily used method, using three pairs of primers (internal, external and loop primers), to get an amplification product which has single-stranded loop regions to which primers can bind without template denaturation [16] at a reaction temperature of around 65°C. Loop-mediated isothermal amplification (LAMP) can be modified to detect RNA targets by the addition of reverse transcriptase enzyme to the reaction. Besides, in RT-LAMP, reverse transcription and amplification of cDNA continue temporarily at a single temperature of around 65°C.

Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) has contributed to the field of virology through the identification of new viruses via popular platforms such as pyrosequencing [17]. In general, map NGS reads the human genome and eliminates host reads after the Blast steps. The remaining data are analyzed to classify into non-human, microbial, or viral integrated sequences.

Electron Microscopy (EM)

Electron microscopy (EM) is one of the primary virus detection methods, especially in unsuspected and unknown agents. This tool can capture live images of cells and tissues with high-resolution. In most cases, the observed morphology can lead to immediate detection at the family level based on particle size, shape, and stability. Transmission electron microscopy (TEM) is a good primary step in virus diagnosis, which targets proteins within the virus structure and is harmless to RNA or DNA genomes. Immuno-electron microscopy (IEM) is also based on the same serological principles as ELISA and can be a useful approach for more virus identification [18].

Cell Culture

The progress in virus isolation using cultured cells has occurred since the addition of antibiotics to the cell culture media, development of chemically defined culture media, and the use of cell-dispensing equipment for preparing replicate cultures [19]. Cell cultures provide large numbers of cells as hosts for the virus and help decrease the use of experimental animals, and hence a lower risk of contamination. Therefore, viruses reach high titers in the cells which are accessible for microscopic examinations. Also, cell cultures are less expensive than eggs and animals [11].

Nanoelectromechanical Devices

High-frequency nanoelectromechanical systems (NEMS) [20] are being considered as new sensors and devices. It has been proved that the selective molecular binding to the surface of nanomechanical oscillators may lead to detecting pathogen viral binding through observing their effects on the natural frequency shift of NEMS devices [21]. This method for virus detection is still in its infancy and needs further development to be more efficient.

USE OF ELECTROCHEMICAL AND NANOBIOSENSORS TO DETECT VIRUS-INFECTIONS ELECTROCHEMICAL STUDIES

Applying nanoparticles (NPs) in combination with electrochemical detection is promising in detecting viruses. A biosensor is an analytical tool applied for the detection of analytes that combine a biological component with a physicochemical detector [22]. The NP-based biosensor is better for detecting pathogenic microorganisms in clinical samples because they are user-friendly and have high specificity, and low costs [23-25].

NanoBiosensor

These devices detect the existence or concentration of a biological analyte, like a biological system or a microorganism, or a biomolecule [26]. Typically, biosensors consist of three components: a part which recognizes the analyte or biological identification part, a signal transducer, and an amplifier or a part which is known as the reader device [27].

Aptamer-Based Detection

Aptamers are artificial single-stranded DNA or RNA oligonucleotides that have a high affinity towards a target, which they are designed to bind to [28]. Aptamer based biosensors or aptasensors, are fabricated by using aptamer as bioreceptors (capturing aptamer/probe) or transducers (signal aptamer/probe) [29].

Optical Aptasensors

1. Surface plasmon resonance or SPR aptasensors, which by evaluating the change of refractivity of a material bound on a surface, measures the resonance of free electrons in the metal films [30]. Typically, the capturing aptamer is immobilized on a metal surface. As the virus binds to the aptamer, the thickness of the metal surface changes, resulting in the alteration of the refractive index. Quantification can be carried out by monitoring the difference in the angle or intensity of light after the virus is bound to the aptamer [31].
2. Calorimetric-based aptasensors use a shift in color to detect a virus. This shift can be seen by the naked eye, or it will require a spectrophotometer [32]. They fall into different groups, for example, nanomaterial-based calorimetric aptasensors [33,34] can use a nanomaterial as support for the aptamer or as a part of the transducer to create the signal with their assistance. Another type is the enzyme-linked aptamer assays (ELAA) which have the same base as ELISA, but use aptamers instead of antibodies as the bio-receptor or the transducer [35].
3. Fluorescent aptasensors, which can be categorized into aptasesnors that respond with fluorescent intensity [36], or the ones that respond with fluorescence polarization [37].
4. Others such as SERS based and CL aptasensors [38].

ELECTRICAL APTASENSORS

- **Electrochemical aptasensors:** Usually in this type of sensor, the aptamer is immobilized on an electrode. In some applications, no enzyme is employed and binding of the immobilized aptamer to the target changes the impedance directly [39].
- **Piezoelectric Transducers:** Some materials such as the quartz crystal microbalance (QCM) have a Piezoelectric effect which is the ability to generate an electric charge in response to applied mechanical stress [40].

METHODS DEVELOPED TO DETECT SARS-COV-2

With the improvement in molecular biological techniques, nucleic acid detection methods have developed at a fast rate and are now considered as one of the best methods for virus detection [41-43]. However, the sample for nucleic acid assays from suspected patients of SARS-CoV-2 is mainly throat swabs [44]. The other method is RT-PCR, which is used for detecting and analyzing SARS-CoV-2 [45]. Polymerase chain reaction (PCR) has numerous advantages, such as fast diagnosis, high specificity, sensitivity, and this ordinary quantitative assay has been considered as the “gold standard” for virus detection [30]. Here below are a number of methods that researchers used for detection of the SARS-CoV-2 virus [8].

PCR-Based Methods

In general, SARSCoV-2 involves mRNA converted into cDNA by reverse transcription, then PCR occurs and the resulting products undergo gel electrophoresis and sequencing to detect the SARS-CoV-2 virus [27,46].

Enzyme-Linked Immunosorbent Assay (ELISA)

Result of the ELISA test for the 91 samples of COVID-19-infected cases indicated that 52 IgM antibodies were positive, the accuracy of the test was 69.0% (87/126) with the specificity of 100% (35/35), and 74 IgG antibodies were positive, the accuracy of the test was 86.5% (109/126) with the specificity of 100% (35/35. The sensitivity for the detection of combined IgM and IgG was 82.4% (75/91) and the healthy controls was negative [45].

qRT-PCR Assay for SARS-CoV-2

The results indicate that these assays are sensitive and specific, allowing for diagnosis of SARS-COV-2, by applying human plasma/serum as soon as 3 days after the symptoms appear. Most importantly, these methods do not need handling of infectious viruses and they can be modified to detect different kinds of antibodies. They claimed that their ELISA method will play a key role in determining the real rate of attack and rate of infection fatality in different human populations, and also in designing the kinetics of the antibody response to SARSCoV- 2 [47].

Microarray-Based Method

The microarray-based method is a quick diagnostic procedure. In this method, at first RNA from the SARSCoV-2 virus produces cDNA which is labeled with special probes during the reverse transcription stage. Afterward, these labeled cDNAs will be loaded onto the solid phase microarrays. Oligonucleotides are fixed on it, which will then go through a series of washing steps, and the free DNAs will be subsequently deleted. In the end, with the special diagnosis probes, SARSCoV- 2 RNA will be

detected. Because of its advantages, this method is one of the popular techniques for the diagnosis of COVID-19 [48].

Newly Developed Methods

The novel methods for detecting RNA are the RNA-targeting clustered regularly interspaced short palindromic repeats (CRISPR) method which makes use of the enzyme, Cas13 [39,50], and immunosensors based on the array of gold nanoparticle-modified carbon electrodes [51].

For more information eager readers are refered to our previus published review paper about this subject [8,52].

CONCLUSION

COVID-19 is an ongoing pandemic disease, and since the progress of infection by the SARS-CoV-2 virus can lead to severe permanent respiratory problems and possible death, there is an urgent need to provide various diagnostic strategies for early detection of the disease.

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