Micro and Nanoscale Technologies for Diagnosis of Viral Infections

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Viral infection is one of the leading causes of mortality worldwide. The growth of globalization significantly increases the risk of virus spreading, making it a global threat to future public health. In particular, the ongoing coronavirus disease 2019 (COVID-19) pandemic outbreak emphasizes the importance of devices and methods for rapid, sensitive, and cost-effective diagnosis of viral infections in the early stages by which their quick and global spread can be controlled. Micro and nanoscale technologies have attracted tremendous attention in recent years for a variety of medical and biological applications, especially in developing diagnostic platforms for rapid and accurate detection of viral diseases. This review addresses advances of microneedles, microchip-based integrated platforms, and nano- and microparticles for sampling, sample processing, enrichment, amplification, and detection of viral particles and antigens related to the diagnosis of viral diseases. Additionally, methods for the fabrication of microchip-based devices and commercially used devices are described. Finally, challenges and prospects on the development of micro and nanotechnologies for the early diagnosis of viral diseases are highlighted.

1. Introduction

The recent coronavirus disease 2019 (COVID-19) pandemic has caused an unprecedented global need for population-wide diagnostic testing. The World Health Organization has emphasized the importance of large-scale testing for severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) to effectively control infection chains and isolate infected people.[1,2] Until a broad immunity is reached or an effective vaccine is developed, diagnostic tests that can safely, rapidly, and cost-effectively identify affected people in the general population will ensure a minimal impact on the economy, public health, and a timely return to the community activities.[2] Notably, such measures to get a rapid and precise diagnosis are necessary as any other medical needs. In general, early and accurate detection of viral pathogens is crucial to treat, control, and prevent epidemics, effectively.

Viruses are the cause of the most common transmittable infection diseases, which have led to major epidemics and pandemics.[3] Although the current COVID-19 pandemic has recently been the center of attention, well-established viruses, such as the human immunodeficiency virus (HIV) or hepatitis viruses have remained global killers with millions of deaths yearly.[4] Emerging viral diseases are also severe threats to the human population and public health,[5] as observed for the avian influenza A (H5N1) in 1997,[6] the SARS-CoV in 2002–2003,[7] the swine influenza A (H1N1) virus in 2009,[8] the Middle East respiratory syndrome (MERS) in 2012,[9] the Ebola
in 2014 to 2016,\textsuperscript{[10]} and the Zika virus (ZIKV) in 2015.\textsuperscript{[10]} Notably, many viruses, which are present only in a few parts of the globe, are now spreading quickly mainly due to the environmental changes and an increased number of travelers. The high prevalence of viral infections has warranted efforts to improve early and accurate clinical diagnostics to achieve effective prevention and clinical management of viral diseases.

Clinical diagnosis of viral infections relies on detecting viral molecules (oligonucleotides or glycoproteins) in biological media. Viruses are tiny (20–400 nm) infectious organisms composed of either DNA or RNA encapsulated in a protein coat or envelope.\textsuperscript{[11]} They infect a living host cell to replicate their genome and other components. The viral envelope is derived from the host cell membrane, and it expresses viral glycoproteins to identify and bind to the receptors present on the host cell membrane. Upon the host cell recognition, the viral envelope fuses with the host cell membrane allowing the viral genome to infect the host. Eventually, the cellular machinery of host cells is taken over by the virus, enabling it to multiply.\textsuperscript{[12]}

In general, viral detection is made by recognizing viral particles, antigens, RNA/DNA, and specific antibodies.\textsuperscript{[13]} Conventional viral diagnosis methods rely on polymerase chain reaction (PCR), solid-phase immunoassays, cell culture, and immunofluorescence. PCR is the gold standard for the clinical diagnosis of viral infection and viral oligonucleotides since the presence of viral oligonucleotide in biological media reflects viral replication and active infection. This method allows the precise quantification of the number of viral copies present in a sample.\textsuperscript{[14]} Similarly, solid-phase immunoassays (SPIs) detect viral antigens in biological fluids quickly and reliably. SPIs are composed of a solid support on which antibodies or aptamers, specific to viral antigens, have been immobilized. They can effectively measure viral antigens with high specificity and simplify the sample processing and interpretation of the results. Similarly, SPIs detect viral antigens in biological fluids quickly and reliably. They can simplify the sample processing and interpretation of results. Still, the virus quantification lacks precision as compared to PCR. Besides, due to their low speed, relatively high cost, and need for trained personnel, these approaches still cannot detect about a third of respiratory viral infections,\textsuperscript{[15]} viral gastroenteritis,\textsuperscript{[16]} and viral encephalitis.\textsuperscript{[17]} Therefore, an accurate and comprehensive viral diagnosis requires technologies that allow point-of-care (POC) applications. These technologies will have a crucial impact on controlling viral infections by rapidly isolating infected individuals and providing the appropriate care. Successful implementation of novel diagnostic tests demands low-cost technologies that are practical, portable, reliable, and accurate. Methodologies implemented for POC tests, notably sample procurement and processing, will benefit from standardized platforms.

The emergence of micro and nanoscale technologies has opened up new possibilities to address the limitation of current diagnostic approaches for improving early, comprehensive, and accurate diagnosis of viral diseases. These technologies create a paradigm shift in the diagnosis of viral infections by taking advantage of miniaturization, automation, practicality, and cost-effectiveness to develop high-throughput screening platforms that comprehensively detect the enormous diversity of mammalian viruses known to infect humans.\textsuperscript{[18–20]}

This review aims to provide micro and nanoscale engineering insights on the diagnosis of viral diseases by covering sampling, sample processing, recognition, enrichment, detection techniques, fabrication of micro and nanoscale devices, and commercialization technologies (Figure 1). We will present how some of the existing challenges could be addressed by nano and microscale engineering approaches. Additionally, some emerging technologies that may contribute to future platforms for the diagnosis of viral infection are discussed.

### 2. Sampling

Current technologies allow for the analysis of various types of samples from the human body. While the blood sample is usually collected for a wide range of laboratory tests, saliva, semen,
urine, sputum, feces, and other bodily fluids and tissues can also be tested.\cite{21} Some samples, including urine, saliva, and feces, can be obtained through natural elimination from the body. However, minor surgery and anesthesia are needed to acquire some other samples, such as cerebrospinal, amniotic, pleural, synovial, peritoneal, or synovial fluid.\cite{22}

The purpose of a particular test often determines the required sample for testing. For instance, a blood glucose test is used for diagnosis of diabetes and low levels of blood glucose in diabetics,\cite{23} while urine glucose is tested when a kidney disorder or a urinary tract infection is suspected.\cite{24} For some diseases, such as HIV, urine, blood, and oral fluid screenings are available, while in some particular applications, a specific type of sampling is recommended.\cite{25} For virological diagnostic proposes, samples collected from the body fluid, which are listed above, are commonly used.\cite{26} Among them, the nose and throat's secretions are the widely used sampling sources for detection of viral infections that affect upper airways, such as influenza or COVID-19.\cite{26,27}

In contrast to the methods mentioned above, the only way for obtaining some particular samples is through breaking the protective layers of the body like skin. The commonly used samples are blood specimens that are obtained through minimally invasive procedures. However, a more complicated process that may require a local anesthetic, such as tissue biopsy, is necessary to collect tissue specimens for diagnosis of viral infections. Other sampling methods may be used to diagnose viral infection from different body fluids including cerebrospinal, bone marrow, amniotic, pleural, synovial, peritoneal, and pericardial fluids.\cite{28}

Another rich source of biomarkers is the interstitial fluid (ISF), which surrounds tissues and cells in the body. However, difficulties associated with accessing and collecting ISF have limited its application in medical diagnosis.\cite{29} As a simple and minimally invasive method, microneedles (MNs) have recently been used to collect ISF through micropores or hollow MNs.\cite{29} However, MNs are not available in the clinic for ISF sampling.\cite{30–32} Sampling from exhaled breath has also been used for cancer, bacterial, and viral detection.\cite{33–36} Devices for exhaled breath sampling are essentially breath collectors that need to be combined with other analytical approaches, such as reverse transcriptase PCR (RT-PCR), to deliver the results.\cite{37} However, a direct breath analyzing device for viral diagnosis has not been developed yet.

### 2.1. MN Patches

MN technology has attracted researchers in the past decades due to its favorable characteristics, including exceptional therapeutic efficacy and painless penetration.\cite{38} For example, MN patches have been studied for several biomedical applications including, transdermal drug delivery,\cite{39} cancer treatment,\cite{40} diabetes monitoring and therapy,\cite{41} and anti-obesity.\cite{42–44} Due to the administrative characteristics of MNs in the form of a patch, they can be used for different diagnostic applications, such as ISF sampling,\cite{29} disease monitoring,\cite{45} or mucus sampling for early etiological diagnosis (e.g., COVID-19).\cite{46}

During the current COVID-19 pandemic, conventional oropharyngeal swabs have mainly been used for viral sampling, which caused a considerable compromise on etiological diagnosis by high “false negative result” rates. In general, MN patch-based swabs have several advantages over plain swabs including higher surface area and penetration depth in the skin, which results in an efficient capturing of high number of viruses. However, fabrication procedure of MN patches is more time consuming and needs more sophisticated equipment compared with regular swabs.

Chen et al. showed that MN swabs can enhance the quantity of virus collection (more specifically SARS-CoV-2) significantly in comparison with conventional oropharyngeal swabs (Figure 2a).\cite{44} They fabricated the MN patches using a 3D-printed poly(dimethylsiloxane) (PDMS) mold. First, a solution containing 2019-nCoV spike/receptor binding domain antibody in phosphate-buffered saline (PBS) was poured into the mold, followed by vacuum and centrifugation. Second, an alginate solution was added to the same mold and left in an oven at 37 °C overnight. The MN patches were peeled off from the PDMS mold at room temperature, and were assembled onto regular swabs. The virus collection of MN swabs was through targeting the spike protein on the virus surface to antibodies in the MN swabs, allowing antigen–antibody interactions during sampling.

Figure 2. Virus sampling using MNs from humans. a) Schematic of a MN/antibody swab providing mucus penetration and virus extraction for COVID-19 detection. The left swap is a regular conventional swap. The right swap is an engineered MN/antibody swab. Reproduced with permission.\cite{44} Copyright 2020, Elsevier. b) Schematic of a MN patch (I) and a wearable electrochemical microfluidic POC chip for diagnosis (II). Reproduced with permission.\cite{45} Copyright 2019, American Chemical Society.
Another study by Yang et al. showed an in situ sampling and quantification of the Epstein-Barr virus DNA from dermal ISF by wearable MN patches.\(^\text{[45]}\) This study did the virus sampling using a functionalized MN patch and quantified the samples using a recombinase polymerase amplification (RPA)-based microfluidic system as a POC testing (Figure 2b). The fabrication of the hydrogel-based MN patch made of gold nanowires (AuNWs@hydrogel) was done through a three-step replication process, followed by the capture-DNA surface’s immobilization of the MN via S–H bond. The MN sampling was primarily based on the virus’s adsorption on the MN surface through the triple helix recognition, and secondly through the methylene blue ligand intercalation. Although the developed technique was promising for capturing Epstein-Barr virus cell-free DNA circulating in ISF, it presented some practical issues. For instance, it was suggested that the RPA reaction might get triggered by the temperature of human skin (≈35 °C), followed by the methylene blue probe intercalation into the RPA amplicons (electrochemical signal output), resulting in low capture efficiency and false detection. It is believed that MN application in virus sampling is still at its early stage and needs further research. MN patches with a low detection limit and high capturing efficiency should focus on reliable early viral disease detection.

### 2.2. Exhale Devices

Despite more than 90 years of research, the transmission modes of common respiratory viruses continue to be the subject of consideration. The recent COVID-19 pandemic increased awareness regarding the early detection of viruses, which showed several unmet demands in our precautionary plans. Highly uncomfortable sampling of respiratory infections and a relative contribution of a wide range of biomolecules to total transmission are major problems in viral sampling. The development of techniques for the collection of viruses from exhaled breath can be considered as a comfortable sampling method to detect respiratory viral infections. There are several techniques to capture aerosolized pathogens from exhaled breath, such as impactors,\(^\text{[46]}\) impingers,\(^\text{[47]}\) filters,\(^\text{[48]}\) condensates,\(^\text{[33]}\) and electrostatic sampling,\(^\text{[49]}\) as listed in Table 1.

Fennelly et al. developed an aerosol sampling system, which was based on two Andersen six-stage cascade impactors for the sampling of exhaled air to study the particle size distribution of infectious aerosols.\(^\text{[46]}\) They demonstrated that this method could quantify infectiousness and transmission of cough-generated aerosols consisting of airborne pathogens, such as M. tuberculosis. In another work, Lindsley et al. used a two-stage bioaerosol cyclone sampler to collect and measure the amount of aerosol particles that consisted of the influenza virus (Figure 3a).\(^\text{[47]}\) The ultrasonic spirometer in the sampling device measured the volume and flow rate of the cough, followed by the flow of the cough into the piston spirometer. The cough aerosols were collected by aerosol sampler when the patient finished coughing. The quantitative RT-PCR was employed to quantify the amount of influenza viral RNA enclosed in the aerosol particles by targeting the M1 matrix gene (a structural protein in the influenza virus).

An electrostatic-based exhale sampling method (Breath ElectroStatic Sampler (BESS)) was developed by Ladhani et al. to detect pathogens using the breath of patients.\(^\text{[49]}\) The developed BESS (Figure 3b) was an electrostatic precipitator consisting of a multi-point discharge electrode composed of electrically connected liquid droplets within a hollow 3D-printed cylindrical cage collector. The study compared aerosol breath samples collected by a nasopharyngeal swab, BESS approach, and gelatin filter (Figure 3c). There was a significantly lower virus

| Aerosol sampler types | Mechanism of action | Advantages and disadvantages | Refs. |
|-----------------------|---------------------|-----------------------------|-------|
| Impactors             | Virus extraction is done based on an elution step of the collected virus from their corresponding solid supports | – Large extracted sample volume<br>– Enormous dilution<br>– Unsuitable for low scale lab-on-chip measurements<br>– Highly complicated and inappropriate for POC applications<br>– High impaction velocity<br>– High-pressure drop<br>– Complex sampling based on a condensation-assisted collection | \[46\] |
| Liquid Impingers      | Working volumes of 5 or 20 mL | – Highly complicated and not suitable for POC applications<br>– Low concentration output | \[47,50\] |
| Filters               | Virus extraction is based on an elution step of the collected virus from their corresponding solid supports | – Large extracted sample volume<br>– Enormous dilution<br>– Unsuitable for low scale lab-on-chip measurements<br>– Highly complicated and inappropriate for POC applications<br>– Noninvasive method<br>– Complex sampling based on a condensation-assisted collection | \[48\] |
| Condensates           | Condensates samples into tiny liquid droplets on a hydrophobic surface | – Noninvasive method<br>– Complex sampling based on a condensation-assisted collection | \[33\] |
| Electrostatic         | Electrostatic attraction between an electret and viral RNA | – Low power consumption<br>– Gentle sampling<br>– Less damaging for pathogens<br>– Can be done directly onto a microfluidic air-liquid interface<br>– Controlling high airflow volume<br>– Consist of a human exhaled flow<br>– Great capture efficiency | \[50,51,52\] |
concentration in the filter-based sampling compared to the swab-based sampling, suggesting a larger number of viruses in the nasopharynx compared to airways. Therefore, this technique showed some detection limitations when there is a low viral load in breath aerosols. On the other hand, there was a lack of efficient virus capture using this technique due to the size-dependent collection efficiency of electrostatic samples. To study exhaled virus aerosols generated during talking, regular breathing, and intense coughing, Huynh et al. made a mask-like sampling device containing an electret as a dielectric material that generates electric fields located opposite of the mouth and nose (Figure 3d).\[53] The electret was removed from the mask after sampling and was added to the RNA lysis buffer. The exhale sampling mechanism was based on the electrostatic attraction between the electret and viral droplets that facilitated the sample collection due to the deactivation of electret by the viral RNA lysis buffer. A high volume of air-flows could be handled by electrostatic-based sampling compatible with human exhaled breath and capture efficiencies were often high (>90% for particles >1 µm).\[54] The electrostatic sampling method also had a low impaction velocity and pressure drop essential for protecting pathogens. Additionally, the electrostatic-based sampling method could obtain 5–10 times higher concentrations of pathogens compared to other discussed methods.\[55] Gouma et al. studied an exhale sampling method to monitor viral infections by interfacing a three-sensor array to capture an exhaled breath and analyze it for the relative content of isoprene (standard volatile organic compounds marker of the flu) and nitric oxide.\[56]

In summary, the current exhaled air sampling methods for diagnosing respiratory viral infections present an up-and-coming and noninvasive method for analyzing and studying viral infections’ pathophysiology. However, further research is needed to detect viruses using the current sampling methods, especially electrostatic breath samplers. The exhale devices can be a promising breath sampling device for non-invasive viral collection during pandemics; however, this technique is not sufficiently developed to be used in clinic.

3. Microfluidic Sample Processing

Conventional methods for diagnosis of viral infection mostly rely on antigen-antibody interactions,\[57] cell culture,\[58] flow cytometry and microscopy,\[59] or detection of nucleic acids (NAS) using PCR technique.\[60] These methods require expensive and sophisticated equipment, a high volume of samples, and experienced operators. Moreover, the detection process is lengthy and the detection threshold is low.\[61] Therefore, low-cost, fast, portable, and user-friendly tools are preferred for improving clinical diagnostics. Recent advances in micro and nanotechnologies have led to the development of various
detection techniques. Among these techniques, microfluidic technologies can address the challenges related to the rapid detection of pathogens. As an alternative technique, microfluidic systems tackle several obstacles in the field of viral detection, providing fast, sensitive, accurate, and autonomous detection platforms in a highly dynamic environment.

Due to the complex nature of the viral infection, which is highly active and affected by physical and chemical stimuli, microfluidic technique can provide a better alternative for the diagnosis of viral infections. Separation and purification of viruses from a complex or low concentrated clinical samples have also been challenging. Recent lab-on-chip technology with a set of microfluidic channels is promising to solve these challenges. Each channel on a microfluidic system is dedicated to a specific function, such as sample preparation, reagent mixing, and detection. This technological advancement allows for the integration of conventional detection methods in a miniaturized chip, suitable for POC testing in viral diagnosis. The system uses a small quantity of samples and provides a precise result in a short time. This type of viral detection is suitable for both clinical and personal uses. Depending on what type of samples are used for viral detection, including blood, saliva, nasopharyngeal swab specimens, or urine, a microfluidic device can separate unwanted moieties and enrich the target. Most importantly, microfluidic systems can provide a promising solution to enrich low concentrations of viruses in samples and thereby increase the chance of detection and diagnosis of viral infections. An example of such microfluidic systems contains channels for fluid and gas, which are separated by a hydrophobic membrane. Evaporation of the fluid while moving through the channel results in the reduction of the sample volume and as a result the fluid concentration increases. Such pre-processing and sample preparation using microfluidic systems can also improve the sensitivity of the detection in low concentrated samples. In this section, we discuss different techniques that have been coupled with microfluidic devices for separation and enrichment of viruses and provide a few examples for each category.

3.1. Physical Techniques

Microfluidics can be employed for viral particle separation/concentration based on physical techniques. Although few studies have integrated sample separation/concentration techniques with a viral detection method in a single microfluidic chip, such integration can significantly enhance the accuracy of results and reduce time and labor costs. Here, we divide physical-based techniques used for the separation/concentration of viral samples into four main categories, including size-based filtration, centrifugal microfluidics, thermal lysis, and hydrodynamic separation.

In the case of size-based separation, a filter-based microchip with a pore size of ≈2 μm was designed to separate HIV spiked from the whole blood by entrapping red and white blood cells into a nucleopore polycarbonate track-etched filter membrane. The filtration process took ≈1 minute with recovery efficiencies of 89.9% ± 5.0%, 80.5% ± 4.3%, and 78.2% ± 3.8% from viral samples with concentrations of 10^3, 10^4, and 10^5 copies mL^−1, respectively. Using a similar concept, Yeh et al. fabricated a portable size-based filtration microfluidic device by a chemical vapor deposition method. They equipped the device with a vertically aligned carbon nanotube (CNT) channel wall with a gap size of ≈117 nm and a porosity of 97%. Using this technique, they were able to capture lentivirus (=128 nm) with an efficiency of 97% in 10 min. In the continuation of the same work, the authors used CNT filter arrays with various porosities shown in Figure 4 for both enrichment and diagnosis of rhinovirus, influenza virus, and parainfluenza viruses using surface-enhanced Raman spectroscopy. Their chip could concentrate the samples by 70-fold and detect the viruses with a specificity of 90% in a few minutes. Another label-free size-based isolation and release microfluidic device was embedded in porous silicon nanowires for virus separation. About 50% of H5N2 avian influenza viruses were physically trapped and enriched in the porous silicon nanowires within 30 min. Approximately 60% of the trapped viruses were released in 24 h by the degradation of the porous silicon nanowire forest.

In centrifugal microfluidics, the entire sample preparation and detection processes occur in a single disc usually made of low-cost polymers. A rotary motor is integrated into the disc to create a spinning motion of fluid without the need for an external pump or high voltage electrical power. Centrifugal microfluidic is specifically useful for lengthy procedures, such as detecting NAs, which require multistep sample preparation. This system can do all sample separation steps, cell lysis, purification, and amplification of NAs in a single integrated disc automatically and sequently. An example of such a lab-on-disc platform is the rotary genetic analysis microdevice integrated with microbead-assisted RNA purification reservoir and reverse transcriptase loop-mediated isothermal amplification procedure (RT-LAMP) chamber. A laser-induced fluorescence was used for the detection of the influenza A virus in this lab-on-disc platform. First, the loaded samples were driven for RNA extraction using capillary valves and a siphon channel. Then, the purified RNA was delivered to the RT-LAMP chamber for amplification by changing the rotation direction. The entire process from loading the sample until the final fluorescence detection of virus took ≈47 min. This microsystem could successfully detect the H1N1 influenza A virus with only 10 copies of RNA with 10-fold higher sensitivity than conventional RT-PCR. To make the device portable, the group replaced the fluorescence detection system with colorimetric-based detection using an immunochromatographic strip. Their new device could successfully detect target H1/M genes of the H1N1 virus in a total of 55 min with a sensitivity of only 10 copies of RNA.

Another example is a compact disk-shaped centrifugal microfluidic chip made of a ferromagnetic disk that spins with external magnetic power and creates Coriolis forces between chamber walls and zirconium/silica-based lysis beads. The bead-beating forces during the spinning procedure create shear forces to samples, resulting in membrane rupture. Simultaneously, Coriolis forces help in further disruption of the sample. This device was used for the isolation of NAs simultaneously for four different samples, including nasopharyngeal aspirate human metapneumovirus (RNA virus), enteroviruses (RNA virus), and adenoviruses (DNA-virus) along with bacterial
spores. The quality of the isolated sample using this microfluidic chip was as good as an in-tube bead-beating standard lysis technique.

In thermal lysis on a chip, viral samples are exposed to elevated temperatures of higher than 80 °C to rupture the virus membrane and release the NAs. The thermal lysis on a chip usually occurs in the same chamber used for PCR to amplify the NAs. This is because the PCR process also requires an elevated temperature of ≈95 °C to desaturate DNA, which can also be used for viral lysis. Lien et al. performed integrated thermal cell lysis and RT-PCR at 95 °C for 5 min using a single chamber on a chip to simultaneously lyse and detect enterovirus 71 and Dengue virus (DENV).

The other physical technique used for viral sample separation and detection in a microfluidic system is using the hydrodynamics of fluids. Zhang et al. engineered a disposable polymeric microfluidic device using 3D maskless xerography method to concentrate the human influenza virus by reducing the fluid volume. The device was composed of a top liquid layer for loading viral samples and a bottom gas flow layer for enhanced evaporation of the liquid in the top layer. A hydrophobic porous membrane was sandwiched between the two layers. At the interface of the membrane pores, gas flow enhanced the evaporation of the liquid sample. The meniscus induced by the evaporation governed the motion of viral particles in the fluid sample and dragged the viral sample along the channel while concentrating it. Using this microfluidic evaporation device, the authors could concentrate viral RNA over 10-fold with a ≈60% recovery efficiency. In another example, Wang et al. used tangential flow microfiltration on the chip to separate/concentrate the HIV (Figure 5).

### 3.2. Chemical Techniques

Chemical-based techniques use chemical reagents, including surfactants, lysis buffers, and enzymes in a microfluidic device to lyse or detect viruses. Different kinds of detergents or lysis buffers can decompose proteins and lipids available in the virus membrane. The most common detergents include sodium dodecyl sulfate, Triton X (100), nonyl...
phenoxypolyethoxyl ethanol, and Tween 20.\textsuperscript{[81]} However, chaotropic agents, such as guanidine thiocyanate or TRIzol Reagent combined with other protein denaturants, such as proteinase K, are more common, especially for viral samples.\textsuperscript{[82]} An example of the enzymatic lysis is the disruption of respiratory syncytial RNA with achromopeptidase dried enzyme, which could lyse viruses in less than 1 min.\textsuperscript{[83]} After the lytic reaction, the enzyme was deactivated using a disposable chemical heater. The extracted RNA could be successfully amplified using RT-PCR. A self-contained portable centrifugal microfluidic disc was used to lyse, amplify, and detect RNA of (H3N2) influenza A virus.\textsuperscript{[84]} The lysis procedure occurred in a lysis chamber on the disc allowing on-demand controlled release of the lysis buffer into the chamber for 10 min, followed by inbuilt shaking to lyse the viral sample. The extracted NAs were transferred to other chambers using an automated rotating set up for further amplifications.

Another technique for chemically assisted NAs extraction is the solid-phase extraction technique, based on interactions between target particles suspended in a liquid phase with the stationary phase.\textsuperscript{[85,86]} This technique is governed by chemical interactions, including hydrophobic interactions, electrostatic interactions, and hydrogen bonds between the NAs and the stationary phase. For example, silica matrices containing positively charged groups were used to electrostatically bind with negatively charged NAs in an alkaline environment.\textsuperscript{[87]} Likewise, anion exchange resins containing highly positive charged diethylaminoethyl cellulose were used for an ionic interaction with negatively charged NAs.\textsuperscript{[86]} However, a desalting procedure using a high salt concentration is needed to further use the extracted NAs. Compared to the silica matrices, the resins can be reused after denaturation. Another common approach utilizes a cellulose-based membrane embedded with lysis buffers and chelating agents like ethylenediaminetetraacetic acid to detoxify metal ions.\textsuperscript{[86]}

For the first time in 1999, Christel et al. integrated solid-phase extraction technology with a silicon microdevice containing pillars to isolate plasmid DNA from \(\lambda\)-phage using a chaotropic salt as the binding reagent.\textsuperscript{[88]} This chip’s efficacy was 50\%, and the DNA with a maximum of 48 kbp could be captured. Despite the device’s low effectiveness, it was a significant achievement compared to other methods at that time. Wu et al. engineered a tetramethylorthosilicate-based sol-gel channel containing micrometer-scale pores using polyethylene glycol as a porogen.\textsuperscript{[89]} This chemistry provided sufficient silicon moieties on the channel’s surface, which resulted in more efficient NAs capturing. The fabricated microchannels could extract \(\lambda\)-phage DNA from the whole blood sample with an efficacy of 85 \(\pm\) 7\%. More recently, Zhu et al. fabricated microfluidic chips integrated with four chitosan-modified silicon dioxide capillaries for isolation and enrichment of the ZIKV RNA from saliva samples.\textsuperscript{[90]} The group also added PCR buffers directly to the device for in situ PCR and amplification of the extracted RNA on the chip. Using this device, an exceptionally low concentration of ZIKV RNA (50 transducing units/ml) could be diagnosed in the human saliva sample.

### 3.3. Magnetic Techniques

One of the popular methods to extract NAs from the lysed virus is using magnetic beads. Magnetic beads are prepared from iron oxide magnetic nanoparticles, which are coated or functionalized with active moieties, enabling them to bind to NAs.
and separating them from other lysed molecules, such as proteins or carbohydrates. Magnetic bead separation system can be coupled with different chip techniques, such as amplification or elution methods, including temperature, concentrated salts, or pH.[76] Furthermore, magnetic beads in fluids can be agitated by an external magnetic field to act as a stir bar for mixing the solution. This technique has often been used to replace the centrifugation in microchips.[78]

Wang et al. designed a specific NAs probe conjugated with magnetic beads to extract HIV RNA.[91] A schematic and photograph of this microfluidic device are shown in Figure 6a, b. The principle of the isolation and detection procedure is summarized in Figure 6c. First, HIV-infected Jurkat T cells were lysed in the reaction chamber by elevating the temperature to 95 °C to release the RNA. The chamber’s temperature was subsequently reduced to 60 °C, making it favorable to hybridize the released RNA with the designed probe on the magnetic beads’ surface. Magnetic beads were then isolated from the unbound moieties using an external magnet during the chip’s washing process. The extracted RNA was further used for amplification in two PCR chambers integrated with the chip. The amplified DNA labeled with a fluorescent dye could be readily detected using an optical detection module. Using a similar approach, Du et al. used magnetic beads conjugated with 4-formyl benzamide in a microfluidic device integrated with air bubbles to capture the Ebola virus RNA.[92] The air bubbles were applied to prevent magnetic beads sedimentation and facilitate bead-solution mixing, which led to a more efficient hybridization of the extracted RNA with the target probe on the beads’ surface. The entire sample lysis and RNA isolation was quick and completed in ≈1 h. Several external magnet-assisted washing steps were performed to remove unbound molecules. For instance, elevated temperature (≈80 °C) was used to release captured RNA from magnetic beads in the presence of a T50 buffer. More recently, Wang et al. fabricated a magnetic-mediated separation of various influenza hemagglutinin types in a microfluidic device.[93] The system had a high detection limit of 3.4 ng mL⁻¹ for H7 and 4.5 ng mL⁻¹ for H9. Lu et al. developed a digital microfluidic device to diagnose the H1N1 virus using aptamer and antibodies-conjugated magnetic beads driven by an electromagnetic field.[94] The droplets of magnetic beads were actuated by a precisely controlled electromagnetic force on a hydrophobic Teflon-coated surface, which automatically completed the entire assay within 40 min. The device limit of detection for the H1N1 virus was 0.032 unit per reaction.

3.4. Electrokinetic Techniques

Electrokinetic methods are contact-free methods and can process biological entities, such as cells, bacteria, viruses, or NAs. They have gained increasing popularity due to high analytical performance, low required reagents, and their ability to integrate multiple technologies in a single instrument. To enrich or concentrate a target in a biological sample, they can also be combined with microfluidic systems.[95, 96] By applying an electric field, high voltage in a chip can lyse viruses and extract their NAs. Park et al. fabricated a microfluidic device containing a silicon probe array with nanoscale tips using an isotropic plasma etching method.[97] Repeated oxidation etching steps were conducted in water in the lysis/hybridization reaction chamber and transferred to the PCR reaction chamber filled with the reagents to amplify the NAs. The NAs are labeled with fluorochrome SYBR Green used as an amplification indicator. (VI) The fluorescence-labeled NAs are detected using an optical detection module. Reproduced with permission.[91] Copyright 2013, Elsevier.
performed to sharpen the tips of the probes. A metal layer was then deposited on the probe, and the entire silicon-based device was attached to a circuit board to provide an electrical connection in each probe. The probe tips were able to capture the vaccinia virus using the dielectrophoresis (DEP) technique. A high electric field \( \approx 10^7 \text{ V m}^{-1} \) was applied to the captured virus by the same probe to lyse the virus further and extract the NAs.

Electrophoresis is the migration of suspended charged particles relative to a fluid by applying an external electric field. However, electrophoresis has limited efficiency and requires extensive and high voltage supply units to generate the energy, which also causes the electrophoresis buffer to overheat. Among various electrokinetic-based methods, DEP is a commonly used approach, which is based on the migration of neutral but polarizable particles in an inhomogeneous electric field. It allows the manipulation of cells or biomolecules according to their size, shape, and dielectric properties. An alternating current is used in the DEP method, and in contrast to direct current, the polarity of electrodes changes once every half-cycle of the alternating current signal. Virus analysis using the DEP method was first shown by Muller et al. in 1996. They investigated the trapping and enrichment of viruses in an aqueous environment using DEP. Iswardy et al. developed a diagnostic platform using a microfluidic DEP for the detection of DENV. They used the microfluidic chip to capture silica beads coated with an anti-flavivirus monoclonal antibody using the DEP force and capturing electrode. Subsequently, the modified beads captured the injected fluorescently-labeled DENV particles using immunoreaction. This detection mechanism, including capturing and guiding a sample, is shown in Figure 7a–c. The platform provided a fast detection of 5 min, with a small sample volume of \( \approx 15 \mu \text{L} \).

The capability to quickly isolate and detect viruses directly in the blood, plasma, serum, and other biological buffers is desired for future diagnostic implementations. DEP-based detection methods appear to have significant potential applications. Maruyama et al. developed a technique to detect and analyze viruses using DEP and optical tweezing. In the platform, a cell was infected by a single influenza virus. The virus was quantified before and after infecting the cell in a microfluidic chip containing the sample and analyzing chambers. The process includes the injection of influenza viruses inside the sample chamber, followed by a concentration of samples from \( 10^6 \) to \( 10^9 \) viruses per \( \mu \text{L} \) using DEP force. The optical tweezers were then used to entrap and transport the selected virus from the sample chamber to the analyzing chamber to evaluate the cell infection.

Figure 7. a) Set-up of the system for DENV detection. Sequential sample b) capturing and c) guiding in the mechanism of detection. The red arrows indicate that the AC voltage is applied. Reproduced with permission. Copyright 2017, Elsevier. d) SWCNT-based immunosensor for detection of the H1N1 virus. Inset: an optical image of PDDA with electrophoretically deposited SWCNTs. Reproduced with permission. Copyright 2014, The Royal Society of Chemistry.
A combination of conventional PCR with DEP method using microbeads has been suggested as an alternative to real-time PCR. Nakano et al. compared the sensitivity of detection of norovirus genome using PCR and real-time PCR methods based on microbeads. They chemically attached biotinylated target DNA molecules to the microbeads and measured impedance changes caused by the DEP-trapped microbeads. Microelectrodes captured microbeads using positive DEP, and the capturing was detected by the recorded changes in the microelectrodes’ impedance. The amplitudes were detected in 20 min using the DEP approach. Ding et al. showed an improved microchannel design for a gradient insulator-based DEP device and the Sindbis virus was successfully captured using DEP force at a low potential.

Influenza viruses spread quickly through the air and are taken into the body via the respiratory tract, resulting in infection in different organs. Therefore, fast and reliable detection of the influenza virus is an urgent need. Singh et al. developed a highly sensitive and unlabeled electrical immunosensor using electrophoretically deposited single-walled CNTs (SWCNTs) to detect the H1N1. They used DEP and electrostatic forces between two gold electrodes to deposit and assemble carboxyl-functionalized SWCNTs on a monolayer of polydiallyldimethylammonium chloride (PDDA). The DEP method was used as a reliable, efficient, and inexpensive method for the deposition of CNTs. Applying the DEP force resulted in the deposition and alignment of CNTs between electrodes. Viral antibodies were immobilized in PDDA-SWCNT channels using biotin-avidin conjugation. The immunosensor was able to detect the virus at a concentration of 1 to 10^6 PFU (plaque-forming units) mL⁻¹ within 30 min of detection time. One PFU mL⁻¹ influenza virus was detected selectively and precisely from MS2 bacteriophages (Figure 7d).

3.5. Immunoaffinity-Based Techniques

Effective treatment of many life-threatening conditions, specifically viral infections, depends on the accuracy and speed of diagnosis. Immunoaffinity-based nano/microfluidic technologies have emerged as powerful diagnostic tools. They are potentially useful for maximizing sensitivity, minimizing sample volume requirements, and producing fast and accurate results. In particular, immunoaffinity-based techniques are sensitive and quantitative methods for the detection of antigens of viruses.

Diagnostic tests for HIV, influenza A, hepatitis C, malaria, and tuberculosis are primarily based on CD4⁺ T-lymphocyte counting. Immunoaffinity-based methods in which an antibody is immobilized in a microfluidic device, can isolate CD4⁺ T cells and viruses from the blood. The CD4⁺ T cell counting is an essential and widely used test in HIV infection and acquired immune deficiency syndrome (HIV/AIDS) management. For this purpose, a microfabricated chip for identifying CD4⁺ T lymphocytes from human blood using immunoaffinity chromatography was introduced by Watkins et al. They immobilized CD4 antibodies in a microfluidic device to detect HIV infection. They reached a low detection limit of 9 cells µL⁻¹ by capturing CD4⁺ T cells in a short time (~10 min). Cheng et al. isolated CD4⁺ lymphocytes from 10 µl of blood for diagnosis of HIV using microchannel surfaces functionalized with CD4 antibody. In another study, Wang et al. presented a microfluidic device in which anti-gp120 was immobilized on a microchannel surface to detect several types of HIV particles efficiently. They showed that HIV subtypes could be captured reproducibly, efficiently, and reliably in the microchip through protein G-based surface chemistry.

To immobilize biomolecules in immunoaffinity-based microfluidic systems, physical adsorption, covalent bonding, and avidin-biotin specific interactions can be used. Lee et al. designed a microfluidic device for H1N1 detection and covered microfluidic channels with gold for the adsorption of fluorescent dye-labeled antibodies. They used a developed gold-binding polypeptide (GBP) for selective immobilization on the gold surface. The immunoreactions between the GBP-recombinant influenza hemagglutinin antigen (GBP-H1a) and its specific antibody in the microfluidic chip were employed to detect the H1N1 virus in blood samples. Antibodies are the most widely used immunoaffinity detection agents because of their high selectivity and ability to target various pathogens. However, they have some disadvantages, such as limited stability under standard environmental conditions, and their production methods are expensive and complicated. Therefore, micro and nano-sized polymeric or magnetic particles can be functionalized as immunoaffinity materials. For example, influenza A virus can be efficiently separated from fluid specimens, such as saliva, serum, and blood in a short time using a microfluidic-based separation technique employing magnetic beads. Ferguson et al. used anti-nucleoprotein antibody-modified magnetic beads to capture the H1N1 influenza virus’s ribonucleoprotein from throat swab samples. For this purpose, they used a 1 cm × 6 cm device that featured three fluidic ports: sample/buffer/reagent input, waste output, and E-DNA product output. To minimize sample loss and achieve fast detection, they integrated the sample preparation with detection testing in a single instrument. The capture, RT-PCR, and ssDNA generation were performed in the sample preparation chamber and the detection was performed in the electrochemical DNA detection cell. They showed that the detection of H1N1 viruses can directly be achieved from throat swabs within 3.5 h with a limit of detection of 10 TCID50, approximately 4 orders of magnitude below clinically relevant infectious doses.

4. Recognition and Enrichment

The presence of biomarkers at low concentrations in samples requires sensitive recognition and enrichment techniques to concentrate the target biomarkers for precise detection. Micro and nanotechnologies can utilize unique properties of nanoparticles and microfluidic-based platforms for the recognition and amplification of NAs. Amplification and generation of multiple copies of NAs is considered a critical step in diagnosis of viral infections with high sensitivity. In this section, the role of functionalized nanoparticles and microchip-based amplification systems in the enrichment of biomarkers are described.
4.1. Functionalized Nanoparticles for Recognition

Viruses are the smallest microorganism existing in different morphological forms and sizes in the range of 20–900 nm.112,113 The nanoscale size and structure of viruses create a challenge for researchers to isolate, visualize, and detect them compared to other microorganisms.114 The discovery of nanoparticles with the matching size of viruses has paved the way for utilizing them in several viral applications.115,116 Different nanoparticles, such as carbon-based nanomaterials (CNTs and graphene), metal nanoparticles (gold, iron, silicon, and silver), and quantum dots (QDs) have extensively been synthesized and functionalized for several biomedical applications, including drug delivery, imaging, sensing, and detection of target of interest.117 Notably, functionalization of nanoparticles through conjugation of biomolecules, such as NAs (DNA or RNA), antibodies, and proteins on the nanoparticles’ surface provides a multivalence capability for nanoparticles.118 This multivalence capability increases the affinity of nanoparticles to attach to multiple receptors on target species. As a result of enhancing binding, functionalized nanoparticles can amplify the detection and sensing of viruses even in low concentrations with high specificity. Herein, we describe how different nanoparticles and their techniques have been used to amplify the target biomarker or enhance the target biomarker’s detection.

Gold, iron, and silver nanoparticles have been utilized to conjugate biomolecules for enhancing target biomarker and receptor interactions.119 These interactions enable us to detect a wide range of viruses. Among them, gold nanoparticles (AuNPs) have extensively been used to detect viruses due to their unique photonic, electric, and catalytic properties in couple with the functionalization of specific biomolecules in a multivalent manner.114 Biomolecules can functionalize AuNPs through chemical conjugations, including thiol reactions and coupling reactions, or physical adsorption methods to enhance binding affinity of nanoparticles. Additionally, biomolecules can be conjugated in situ during the synthesis of AuNPs.119 For example, Tang and Hewlett coupled biotinylated secondary anti-p24 antibody to streptavidin, which subsequently coated AuNPs.120 They showed that the antibody-coated AuNPs could detect p24 antigen for HIV type-1, with a significantly low limit of detection of 0.1 pg mL\(^{-1}\). In another study, Chang et al. proved that chemical adsorption of hemagglutinin antigens connected to protein A on spherical AuNPs could enhance the detection of hemagglutinin antigens on the H1N1 virus by 1000 folds.122

Functionalization of biomolecules on various sizes and shapes of nanoparticles could also affect the detection of viruses as an increase in surface to volume ratio enhances the local contact interaction. For instance, anti-p24 monoclonal antibodies were functionalized on different sizes of spherical AuNPs.122,123 Antibodies coated on 20 nm of spherical AuNPs exhibited 200 folds higher detection compared with 30 nm AuNPs. Zeng et al. demonstrated the effect of nanoparticles’s shape on the hepatitis B surface antigen detection using QDs-gold nanorods (GNR)-based fluorescence resonance energy transfer technique (FRET) nanosensor.124 Hepatitis B surface antibody was immobilized on GNRs (GNR-antibody) as the FRET acceptor. In addition, the hepatitis B surface antibody was tagged to red/green color QDs (QD-antibody) as the FRET donor. The addition of analyte Hepatitis B antigen to a solution containing the GNR-antibody and QD-antibody caused immunoreaction between two nanoconjugates. As a result, the photoluminescence of QDs was quenched by the GNR. The amount of quenching during the FRET process was proportional to the number of detected antigens. This methodology increased the detection limit to 9 ng mL\(^{-1}\). This increase could be attributed to high self-assembly property of rod-shaped nanoparticles, enhancing the interaction between the antibodies and antigens.

In addition to AuNPs, iron and silicon nanoparticles, QDs, CNTs, graphene, and hybrid nanoparticles (AuNPs-GdSe/ZnS) have been functionalized with biomolecules via phosphate, silane linked with perfluorophenyl azide, and thiol coupling linkers. The selection of nanoparticles depends on the mode of methods to detect viruses. For instance, iron nanoparticles possess inherent magnetic properties. The functionalization of iron nanoparticles with antibodies enables us to detect specific antigens present on the viral target surface. Due to magnetic attraction, these iron nanoparticles tend to aggregate. Therefore, a detectable change in T2 magnetic relaxation of protons occurs in the surrounding media.125 QDs exhibit excellent fluorescence property and functionalization with antibodies that aids to image viruses.126 Likewise, silicon nanowires, graphene, and CNTs have high optical or conductive properties that can be utilized to detect viruses based on the change in those properties.127–130

Figure 8. General schematic representation of functionalization of nanoparticles.
Knowledge acquired in the functionalization of antibodies on nanoparticles has given a promising way to detect SARS-CoV-2. Moitra et al. functionalized antisense oligonucleotide, specific for the N gene of SARS-CoV-2, on AuNPs by thiol modification.[133] Functionalized AuNPs not only detected the SARS viruses by the naked eye but also enhanced the limit of detection to 0.8 ng μL⁻¹. Similarly, graphene and magnetic nanoparticles were functionalized to detect COVID-19.[130,132] In a recent study, Seo et al. coated graphene sheets with a specific antibody against SARS-CoV-2 for COVID-19 diagnosis.[134] The functionalization of a device by the antibody was achieved through a physical adsorption method using 1-pyrenebutyric acid N-hydroxysuccinimide ester as an interface coupling agent. Nasopharyngeal swab specimens of COVID-19 patients and antigen of protein were used to study the performance of the biosensor. They could detect SARS-CoV-2 with 1 fg mL⁻¹ concentration using a field-effect transistor sensing method. Additionally, this COVID-19 graphene-based field-effect transistor sensor could distinguish the SARS-CoV-2 antigen from MERS-CoV antigen.

4.2. Nucleic Acids Amplification

Detection of infectious organisms and their genetic markers is considered an essential method for diagnosing viral infections. However, detection and analysis of NAs from biological samples require multi-steps, including sample preparation and extraction of NAs, amplification of NA, and their detection.[135] In most cases, NA concentration in extracted samples is not high enough, leading to an insufficient sensitivity for detection. Therefore, NA's amplification is a crucial step for accurate diagnosis of viral diseases as it increases the concentration of NAs to a detectable level.[135,136] Thermal cycling amplification methods, such as different PCR techniques and isothermal amplification methods, are the most common techniques developed for the amplification of NAs. Based on temperature-dependent reactions, PCR techniques require a bulky and expensive thermocycler to control the heating process at three temperature steps. This results in a simplified molecular biology protocol while increasing the cost and complexity of PCR machines.[135] Isothermal amplification techniques have attracted significant attention since they are more straightforward and more energy-efficient as the reaction is controlled at one temperature.[137] Although these techniques without the need for thermal cycling reduce the heat control complexity, they are accompanied by a more complex molecular biology protocol. Additionally, they still require a minimal heating process and source of energy.[138]

Microfluidic technique has demonstrated promising miniaturization applications and has enabled us to scale down the NA amplification devices with less complexity. Microfluidic devices with small sizes can provide faster and more efficient heat transfer.[139] However, there is still a need to integrate all complicated steps of NA amplification in a single microfluidic device. Additionally, the development of a single microfluidic chip for DNA analysis that is capable of extraction, amplification, and detection of NAs in a short time is still challenging. However, advances have been made in this area.[140] Several types of thermal and isothermal NA amplification methods with high sensitivity have been introduced using microchip and microfluidic systems,[140] which will be discussed in the following section.

4.2.1. Thermal Cycling Amplification

PCR, invented in 1986, is the most established molecular technique for NA amplification.[141] Each PCR cycle involves three thermal steps, which include denaturing of double-strand DNA into single-strand DNA at a denaturing temperature of 95°C, annealing of primers to the denatured strands at an annealing temperature of ≈55 °C, and elongation of primers using a thermostable DNA polymerase enzyme at an extension temperature of 72 °C.[142] The number of DNA molecules is doubled in each PCR cycle, which should be repeated to reach enough detectable DNA copies. Typically, about 40 thermal cycles are required to achieve a sufficient amount of DNA.[142]

In addition to the original endpoint detection PCR, qPCR and RT-PCR have widely been used in viral disease assessments.[143] The addition of an intercalating fluorescent dye or a fluoroescently labeled probe in qPCR method allows the real-time quantification of the DNA sequence by recording the fluorescence signal using a detector after each thermal cycle during the synthesis process.[144] In addition to qPCR, RT-PCR has been suggested for complementary DNA (cDNA) amplification in virus detection using RNA as the template.[145] The RNA is activated by reverse transcriptase to generate cDNA and then PCR is carried out to amplify the DNA fragments using the cDNA. An integrated microfluidic RT-PCR model was developed to detect DENV serotype 2 and enterovirus (EV) 71 by Lien et al.[79] They used magnetic nanobeads conjugated with an antibody that selectively captured the target virus, followed by transferring the bead-bound virus to the reaction chamber of RT-PCR. The RNA was then released during thermal lysis, and cDNA was synthesized and amplified using the RT-PCR technique (Figure 9a).

qPCR and RT-PCR techniques can be designed on PCR chips substrates made of PDMS, polymethyl-methacrylate, silicon, or glass. PCR chips have several advantages over traditional PCRs, including high surface area, which leads to a higher rate of heat transfer and a more uniform internal temperature providing better control over the reactions. The small size of the PCR chips makes them portable and small amounts of samples and reagents needed for the analysis significantly reduce the cost of the process.[145]

PCR chips can be classified into two categories in terms of thermal cycling in devices (i.e., flow PCR chips[146] or stationary PCR chips[139]). The stationary PCR chips are composed of a single or multiple stationary PCR chambers. The PCR sample in the stationary PCR chips is injected into a PCR chamber, and the temperature of the reaction chamber is switched between three different temperatures during each cycle.[139] In this method, the whole chip and the sample will undergo temperature changes, which results in a long thermal cycling process. It also requires complicated temperature controlling devices. However, in flow-PCR chips, a continuous flow containing the sample repeatedly circulates through fixed temperature zones

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Figure 9. a) Photographs and illustration of an RT-PCR chip including micro temperature, bead collection, and microfluidic control modules. Reproduced with permission.[79] Copyright 2007, Elsevier. b) RT-PCR device for operating four reactions at the same time to detect the Ebola virus and the recorded PCR amplification curve for Ebola RNA and GAPDH gene (black) as the positive control, GAPDH RNA (red), Ebola RNA (green), and the no-template control (NTC) (blue). Reproduced with permission.[152] Copyright 2016, American Chemical Society. c) RT-PCR reaction chip containing four zones, Zone 1: mixing zone, zone 2: reverse transcription zone, zone 3: polymerase activation zone, and zone 4: thermal cycling zone. Reproduced with permission.[153] Copyright 2018, Springer Nature. d) Strategies used to employ a microfluidic chip, integrated with LAMP method to detect multiple respiratory viruses. Reproduced with permission.[169] Copyright 2018, The Royal Society of Chemistry. e) Schematic illustration of visual readout strategies of digital single-molecule amplification using digital LAMP amplification method and an unmodified camera phone. Reproduced with permission.[39] Copyright 2017, American Chemical Society.
in the microfluidic device with an inlet and collection outlet. In flow PCR chips, only the PCR flow is heated and cooled resulting in less energy consumption.[146] Flow PCR chips offer more flexibility as they can change the sample motion and flow rate, thus controlling the number and duration of each temperature cycling process.[147] Additionally, the flow of PCR chips can be designed based on oscillatory[148] and closed-loop flows.[149,150]

In oscillatory PCR chips, the sample flows back and forth into different temperature zones,[148] while in a closed-loop PCR chip, the sample moves around a closed cycle.[149,150] The first continuous flow PCR device was designed based on the capillary force. However, it was not a chip-based device, which hindered its further application in an integrated microfluidic device.[151]

An RT-PCR handheld device was designed for the detection of RNA of the Ebola virus, which was composed of four chambers to perform four stationary PCRs simultaneously (Figure 9b).[152] A microfluidic chip with a long channel and different temperature zones was also developed to do RT-PCR for Ebola virus detection (Figure 9c).[153] The RNA was converted to cDNA in the first temperature zone between 50 °C and 57 °C, with subsequent PCR amplification of DNA and detection of DNA using a fluorescent system. A rotational PCR chip with an annular microchannel and AC magnetic field that drives flow through the channels was introduced by West et al.[154] Wang et al. developed a micro-oscillating PCR chip at three temperature zones, which was applied for amplification of DNA of human papillomavirus (HPV) with a processing time of 15 min.[155]

Droplet-based PCR (dPCR), which is based on the separation of the sample into many portions to get one or non-DNA copy in each piece, has attracted much attention due to its higher resolution and ability for detection of both DNA and RNA.[156] In Isothermal Amplification

4.2.2. Isothermal Amplification

Although thermal PCR amplification techniques have broadly been employed for NA amplification, they require a bulky thermocycler to control heating process and three-hour amplification.[140] It is hard to miniaturize microfluidic devices based on thermal PCR amplification techniques for POC applications. Additionally, high price and need for trained operators limit the usage of mini thermocyclers in POC applications.[162] To address these issues, advanced isothermal amplification techniques have been developed to provide low-cost and portable systems, which do not require different temperature zones and complex integrated equipment.[137,163] The isothermal amplification techniques work at a constant temperature between 37 °C and 65 °C, which make them simpler compared to thermal PCR amplification techniques leading to easier maintenance and portability. Additionally, the amplification of target gene under isothermal conditions reduces the detection time to less than 1 h, which is considerably lower than thermal amplification techniques.[164] Moreover, the usage of four or six primes in isothermal techniques compared to two primes in thermal techniques significantly enhances the specificity of thermal techniques by recognizing more regions of target NAs.[165] LAMP and RPA are the most widely used isothermal amplification techniques, which are suitable for POC applications.

LAMP technique operates at a constant temperature of 65 °C, and DNA is synthesized and self-recycled using different sets of primers and DNA polymerase.[166] This technique is based on primer annealing and auto-cycling strand displacement. LAMP technique with the capability of amplifying 10⁶ copies in one hour is considered a highly efficient amplification technique.[167] This technique can be integrated with a reverse transcriptase technique under the same working condition using RNA and DNA targets. Additionally, this technique has a high sensitivity, which makes it possible to detect viruses, such as the hepatitis B virus (HBV) at detection limits as low as 60 copies.[168] Automated microfluidic LAMP systems have been developed to reduce the risk of contamination and false-positive signals during manual operations. The detection time in LAMP microfluidic devices is 15–40 min, and the reaction volume is 5–50 mL.[139] The LAMP microfluidic systems can be integrated with different detection methods, such as the commonly used fluorescence detection method. For example, Fang et al. introduced an octopus-like multiplex microfluidic device based on DNA amplification using a LAMP technique integrated with fluorescence signal detection.[168] This technique could analyze multiple genes and differentiate three human influenza A sub-strains in 0.5 h, with a detection limit of less than 10 copies µL⁻¹ for 2 µL of the sample. An integrated real-time LAMP microfluidic system was developed by Wang et al. for diagnosing cDNA from H1N1, H3N2, H5N1, and H7N9 influenza viruses.[169] The microchip was made of eight microfluidic array chips to extract DNA, followed by a real-time LAMP system for the amplification and detection of viruses (Figure 9d).

RPA is another isothermal amplification operating at low temperatures from 37 °C to 42 °C, thus reducing power consumption compared to the thermal PCR and LAMP methods.[170] It amplifies NAs using a recombinase, which binds to single-stranded NAs, single-strand DNA binding proteins, and a strand displacement DNA polymerase.[171] This amplification method is faster than PCR and LAMP and operates at low reaction temperatures, thus reducing sample evaporation in the system.[170] Additionally, low operation temperatures in RPA techniques enable the development of paper-based devices as they do not require extensive thermal facilities or cycle control.
systems. Paper-based microfluidic systems utilize paper as the solid matrix to control the fluid flow. Magro et al. introduced a paper-based microfluidic device using RT-RPA amplification of RNA, which was successfully used to detect the Ebola virus.[172]

In addition to digital PCR, digital isothermal amplification techniques, such as digital LAMP and digital RPA have been suggested for ultrasensitive detection of viral infections, such as HIV and hepatitis C infections.[139] Rodriguez-Manzano et al. developed a SlipChip based on a digital LAMP amplification method.[139] After the single-molecule amplification, the amplification indicator dyes were detected using an unmodified camera phone. The resulting image was analyzed quantitatively using ratiometric image processing (Figure 9e). In this method, phage lambda DNA was used as a model and hepatitis C viral (HCV) RNA as a clinical target.

5. Detection Techniques

Sensing techniques for detecting target biomolecules should be sensitive, low-cost, and easy to use. Micro and nanotechnologies offer various techniques for developing biosensors and microchip-based systems, which can be readily utilized as POC platforms with a low amount of sample volume. Miniaturized biosensors can be classified based on the nature of the detection method, either optical or non-optical detection strategies, each with its drawbacks and advantages. The integration of nanoparticles within these platforms can also enhance the detection signaling. In this section, the application of nanoparticles and microchip-based systems, such as microfluidic and microarray platforms, for optical and non-optical sensing are described.

5.1. Nanoparticle-Based Techniques

Nanoparticles with a high surface-to-volume ratio enable rapid, real-time, and sensitive diagnosis of various viral infections. The sample volume required for testing is tiny, resulting in nanodiagnostic approaches being low-cost and straightforward. Many nanoparticles, such as metallic and non-metallic nanoparticles, have been used to detect infectious diseases (Table 2). Gold, silver, iron oxide (magnetite), zinc oxide, titanium dioxide nanoparticles are the most commonly used metallic nanoparticles for viral detection.[172] QDs have also been utilized for labeling multiple biological targets due to their advantages, including high brightness and extraordinary photostability.[172] Besides, graphene-based nanomaterials with high mechanical strength, conductivity, surface area, and surface functionality have been used for biosensing of various biomarkers NAs and viral diseases.[139] These nanoparticle-associated detection methods are based on chromatic or electrochemical changes in the existence of target virus particles or antigens. Here, we discuss the role of various nanoparticles in the detection of multiple viruses, such as HIV, hepatitis, influenza virus, herpes simplex virus, and SARS-CoV-2. In addition, we also discuss nanodevice-based detection of viral diseases to meet the increasing demands to treat patients with viral infections.

Metal-based nanoparticles can interact with electromagnetic radiation and emit strong signals.[174] Color change-based detection of AuNps, conjugated with proteins, antibodies, and aptamers, has been used to detect various diseases.[175] Exceptional optical and chemical properties of AuNps have also allowed them to be used with main plasmonic biosensing approaches, such as Rayleigh scattering, localized surface plasmon resonance (LSPR), surface-enhanced Raman scattering, fluorescence enhancement, quenching triggered by colorimetry, and plasmon changes.[176] Plasmonic as an important branch in nano-optics has widely been applied in biosensing with improved sensitivity for refractive index changes as well as enhanced light/matter interactions. Thus, these plasmonic biosensing approaches can provide label-free, signal enhanced, and real-time sensing for detection of viral diseases at the molecular level.[176] Changes in the resonance intensity (Rayleigh scattering), refractive index LSPR, vibrational modes (surface-enhanced Raman scattering), excitation and decay rates (fluorescence enhancement), degree of color (quenching triggered by colorimetry), and plasmonic resonance (plasmon) can be used to detect mixing AuNps with specific viral diseases. Ag nanoparticles (AgNps) are capable of electron transfer to the electrode surface and were used to detect infectious diseases in an aqueous solution using surface-enhanced Raman scattering.[177] Moreover, AgNps have been combined with AuNps in the forms of alloys or core-shell nanoparticles for biosensing.[178] For instance, Au nanorods were used for the detection of single-base-mismatch HIV-1 virus DNA via second-order nonlinear optical properties.[179] The intensity of hyper-Rayleigh scattering increased when the gold nanorods screened the HIV viral DNA sequence. Besides, AuNps were developed to detect HCV RNA using a similar hyper-Rayleigh scattering method.[180] Surface-enhanced Raman scattering was used to detect HIV gene-related DNA using multilayer metal-molecule-metal nanojunctions between AuNps,[183] DNA-conjugated AuNps,[182] and multicolor DNA-mediated Au–Ag nanomushrooms.[183] Furthermore, paper-based POC nanodiagnostic systems were developed using antigen-conjugated Au colloids for the detection of HBV.[184] and multicolored Ag nanoparticles for the detection of the Ebola virus.[185] Detection of COVID-19 using this nanodiagnostic system was also developed using Au nano-islands by the combination of the plasmonic photothermal effect and LSPR sensing transduction.[186]

Metal oxide particles, including iron oxide nanoparticles (maghemite (γ-Fe₂O₃), hematite (α-Fe₂O₃), and magnetite (Fe₃O₄)) have widely been used in the field of biomedicine. Their sensing mechanism is based on improved separation and detection of target antigen-bonded nanoparticles in a magnetic field, mainly using magnetic resonance imaging.[187] Combinations of metal and metal oxide nanoparticles can also be used for detecting various viral diseases. For example, the HBV was detected using biotin–DNA probes and streptavidin-based magnetic nanoparticles through nonfaradic electrochemical impedance spectroscopy.[188] Moreover, iron magnetic particles were used to isolate the influenza A virus H9N2 subtype, and then the isolated virus could be electrochemically detected using AuNps.[189] Similarly, the combination of AuNps and magnetic
beads was used for colorimetric detection and quantification of HBV and hepatitis E virus (HEV).[190]

QDs have widely been used as one of the essential sensing materials to detect various infectious diseases. They have significant advantages of broad absorption, narrow emission properties, and a high surface-to-volume ratio, allowing them to be used for excellent biosensing applications. For instance, a CdSe/CdS/ZnS QD as the fluorescent dye was developed to detect the influenza A virus.[126] The QDs were conjugated with latex and then antibody (QD+latex+antibody), showing high stability, sensitivity, and response time. CdZnSeS/ZnSeS QDs and AuNps have also been developed as a biosensor to detect the fluorometric virus using LSPR, where a customized peptide can control the distance between AuNPs and QDs.[191]

Carbon-based nanomaterials, such as graphene, CNTs, carbon dots, and graphene QDs have also been employed

| Table 2. List of nanoparticles utilized for detection of different viral infections. |
|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Particle types  | Particle composition | Particle structure | Detection method | Target species | Refs. |
| Metal nanostructures | Gold | Nanorods (120 × 45 nm) | Hyper-Rayleigh scattering | HIV-1 virus DNA | [179] |
|                  |     | Nanoparticles (5 to 110 nm in diameter) | Hyper-Rayleigh scattering | HCV virus RNA | [180] |
|                  |     | Multilayer metal-molecule-metal nanojunctions (14 nm in diameter) | Surface-enhanced Raman scattering | HIV-1 virus DNA | [181] |
|                  |     | DNA-conjugated nanoparticles (30–40 nm in diameter) | Surface-enhanced Raman scattering | HIV-1 virus DNA | [182] |
|                  |     | Colloids | Visible colored band in strips (POC) | HBV surface antigen | [184] |
|                  |     | DNA-conjugated nanoislands | Surface plasmon resonance (POC) | SARS-CoV-2 | [186] |
| Silver           | Nanoparticles (=30, 41, and 47 nm for orange, red, and green color, respectively) | Visible colored band in strips (POC) | DENV NS1 protein, yellow fever, and Ebola viruses | [185] |
| Gold+Silver      | Gold–silver nanomushrooms (=100 nm in diameter) | Surface-enhanced Raman scattering | HAV (Hepatitis A Virus)/HBV DNA | [183] |
| Metal oxide nanoparticles | Iron oxide | Nanoparticles coated streptavidin nanoparticles (=237 nm in diameter) | Electrochemical analysis | HIV & HBV DNA | [188] |
|                  |     | Iron magnetic nanoparticles (=several 100 nm in diameter)-virus-AuNPs (=14 nm in diameter) | Chrono-amperometric detection | Influenza A virus | [189] |
|                  |     | Beads (=1.05 μm in diameter) combined with AuNPs (=13 nm in diameter) | Colorimetric detection | HAV & HBV DNA | [190] |
| QDs              | CdSe/CdS/ZnS QDs | QD + latex + antibody (=181 nm in diameter) | Fluorescent immunochromatographic test | Influenza A virus | [126] |
|                  | CdZnSeS/ZnSeS QDs | CdZnSeS/ZnSeS QD-peptide-AuNPs nanocomposite (=57 nm in diameter) | Localized surface plasmon resonance | Influenza virus | [191] |
| Graphene-based nano-materials | Graphene | Graphene sheets on field-effect transistor biosensor (=1.530 nm of surface roughness) | Electrical measurement | SARS-CoV-2 spike proteins | [133] |
|                  | Reduced graphene oxide | Polyamidoamine-functionalized reduced graphene oxide (=1.56 to 3.19 nm of surface roughness upon introduction of E-proteins) | Surface plasmon resonance | DENV E-proteins | [192] |
| MWCNTs           | Au/iron-oxide nanoparticle-MWCNT hybrid nanomaterial (=100–200 nm in diameter of CNTs & up to =200 nm in diameter of nanoparticles) | Linear sweep voltammetry | Influenza virus & norovirus DNA | [193] |
as candidates for biosensors of infectious diseases using electrochemical impedance spectroscopy, surface-enhanced Raman scattering, and fluorescence enhancement. For example, a field-effect transistor biosensor was developed using graphene nanosheets for sensing SARS-CoV2 spike proteins in clinical samples. A surface plasmon resonance sensor was fabricated using polyamidoamine-functionalized reduced graphene oxide to detect DENV. Additionally, Au/magnetic-nanoparticle-decorated CNTs were used for creating a biosensing platform. In this work, the thiol-functionalized and DNA-conjugated AuNPs could detect target DNA in the presence of other viruses, including the ZIKV and influenza virus, using DNA sequence mismatching.

5.2. Microchip-Based Techniques

Optical assays based on dyes, fluorescence, luminescence, and metal indicators have increasingly been used for sensitive detection of NAs of viruses with high sensitivity through a visible color change. Optical biosensors including fluorescence, nanoplasmonic, and photonic crystal-based biosensors can be further integrated with microchip based devices to detect viruses. Fluorescence microscopy is the most prevalently used optical method for the diagnosis of diseases. In this approach, fluorescent markers are conjugated with antibodies for specific tagging of proteins or cellular components. Flow cytometry is a well-established method for detecting, separating, and counting cells according to their fluorescent markers, using a laser beam. This method can also be used for the detection of viral NAs. For instance, microparticles coated with anti-digoxigenin and antidinitrophenyl were employed to capture PCR products by using flow cytometry. The primer system was able to detect all known HIV-1 subtypes, including subtypes O (outlier), N (nonmajor and nonoutlier), or novel circulating recombinant forms, with a sensitivity of more than 50 to 1 million copies per PCR. The current flow-based NAs technology crosslinks probes with fluorophores, using HIV-1-specific transcription-mediated amplification for sorting and characterizing RNA or DNA. It was able to detect infected cells with a sensitivity of one in a million. However, this technique is costly and cannot be used for high-throughput applications.

Microfluidic technology can provide an on-a-chip platform to miniaturize the diagnosis methods, enabling direct counting of fluorescently labeling cells. In this regard, a bioactivated nanochip device, containing waste reservoir and reagent storage equipped with a portable single wavelength epi-fluorescent microscope was designed to capture and image QD-labeled CD4 cells. Recently, a rapid and sensitive virus detection system by combining microfluidics and optical biosensor was developed to diagnose human adenovirus. In this system, the viral DNA extraction was enabled through a microchannel using a non-chaotropic reagent and a disposable thin film. This virus detection platform removed the need for complex instruments and enabled 100 folds higher sensitivity than RT-PCR. The virus diagnostic systems’ sensitivity was validated in 13 clinical specimens, and the biosensor was able to detect 10 copies of human adenovirus in 0.5 h. A portable POC diagnosis of viruses during pandemics is of high importance. Optical sensors can also be used for high-resolution imaging of viruses in POC applications. Wei et al. designed an optomechanical device for fluorescent imaging of a single nanoparticle and virus using laser diode excitation and a smartphone. This smartphone-enabled microscopy facilitated the detection of isolated fluorescent particles (100 nm) and fluorescently labeled-human cytomegaloviruses.

Nanoplasmonic biosensing is another optical technique based on optical resonance for conduction electrons oscillating in metals, which has been used in microchip based devices for viral detection. In plasmonic systems, metal structures inside nanometric volumes enable entrapment of light, leading to an increase in near-field signals. Commonly used plasmonic optical-based biosensors include planar surface plasmon resonance (SPR) and LSPR, based on confined AuNPs. SPR and LSPR are nondestructive methods that can detect single molecules and sense refractive index fluctuations in a target binding with nanoparticles or a film. SPR and LSPR are available in the market and offer label-free and sensitive real-time biosensing technology. They have shown great potential to capture different viruses, such as SARS, MERS, H1N1, and influenza. Recently, Qiu et al. designed a dual-functional LSPR-based biosensor for sensitive diagnosis of SARS-CoV-2 with a detection limit of $0.22 \times 10^{-12}$, enabling high efficiency in sensing a specific target in a multigene solution. The plasmonic photothermal effect enhanced thermoplasmonic NAs hybridization, promoting the sensor’s capability to discriminate two similar gene sequences (Figure 10a).

The LSPR can also be combined with microfluidics and nanofabrication techniques to enable the development of susceptible and portable viral diagnosis devices. A benchmark in the design of label-free microfluidic-based LSPR for biosensing DNA-enzyme interactions in real-time was developed recently by Roether et al. This microfluidic LSPR chip was created by bonding a PDMS channel with a nanoplasmonic substrate, following by functionalization with single-stranded DNA template (T30) and spacing with hexanediol. Then, the DNA primer (P8) was attached to T30, following by the elongation of the second strand using DNA polymerase assembling nucleotides from the surrounding fluid. The device enabled an in situ reaction for all steps inside the microfluidic channels at room temperature. The nanoplasmonic substrate was fabricated by using a nanofabrication technique to develop a stack of mushroom-like nanostructure surfaces with an average distance of 19 nm with the form of silicon dioxide stems and gold caps with the sizes of $\approx 40$ and $\approx 22$ nm, respectively (Figure 10b,c). They showed that the immobilized DNA and hexanediol molecules in the LSPR sensor surface could be precisely detected with this biosensor. Nanoplasmonic biosensors have also been used to measure HIV-1 and its subtypes from patients’ whole blood. In another study, plasmonic surfaces were modified in several layers, including a poly-L-lysine layer, a gold monolayer, and a target-specific antibody for antibody immobilization to capture the HIV-1. This platform was used for clinical detection of HIV-1 with high sensitivity (less than 100 copies mL$^{-1}$ in a mixture of HIV-1 subtypes). This system has been used for other infectious diseases and has recently been advanced to a portable device with a potential for being used in resource-limited conditions.
The photonic crystal sensing mechanism is another optical method based on recording a shift in resonance wavelength corresponding to a target analyte’s concentration in response to a biomolecular interaction on the photonic crystal surface. Photonic crystal-based biosensors have been used to detect cells, proteins, nucleic materials, and pathogens (Legionella pneumophila bacteria and human influenza virus). In a study, an anti-gp120 antibody-functionalized photonic crystal surface was developed and evaluated to detect the HIV-1 virus at low concentrations in the range of 10^4–10^8 copies mL⁻¹ in serum (Figure 10d,e). In this approach, the HIV-1 virus from serum was captured and detected using a titanium dioxide-coated 1D photonic crystal biosensor. Also, efforts have been made to accommodate this technique for portable diagnosis systems for resource-limited applications based on smartphone incorporated photonic crystal biosensors. Research is continuing.
to diminish the size of photonic crystal systems, such as using microfluidic channels integrated with photonic crystals for the detection of biotargets. These efforts show that utilizing microfluidics and smartphones with photonic crystals offers portable optical biosensors for viral disease detection.

Electronic sensors have been considerably developed on POC devices as the microelectronics can easily be integrated with miniaturized chips. The conductometric biosensor is an essential analytical tool that contains a transducer to measure the conductivity of a thin electrolyte layer close to the electrode surface. It can reflect specific biological recognition reactions. The biosensor can be used as a sensing platform to detect various pathogenic viruses, including COVID-19, H1N1, and ZIKV (Figure 11a). A conductometric biosensor does not require a reference electrode. It can operate at a relatively low-amplitude alternating voltage, and as a result, Faraday processes on the electrode surface can be avoided. Patolsky et al. developed a microfluidic chip-based device based on nanowire arrays as the field-effect transistors for real-time electrical detection of single virus particles. This technique could detect different viruses in parallel and treat the samples without any extra purification, which means it can exceed the capabilities of the current commercial approaches such as PCR.

Electromagnetic (magnetic) is another popular technique that holds a promising application for the diagnosis of viral infection by integrating microfluidic or microarray devices. Lu et al. proposed a structure-free digital microfluidic chip to diagnose the H1N1 virus by utilizing electromagnetic forces and magnetic beads (Figure 11b). The limit of detection of this technique was estimated to be 0.032 hemagglutination units comparable to the latest rapid influenza detection tests. The overall assay response time on the magnetic digital microfluidic chip was as low as 40 mi, which might be considered as a rapid diagnosis. Sharma et al. reported an integrated platform for the detection of natural DNA extracted from HEV by utilizing the magnetic barcoding technique with high selectivity and specificity. This platform was also employed to detect varieties of bacteria, such as Listeria (Figure 11c).

Piezoelectric biosensors are known to be mass-sensitive detectors with an oscillating piezoelectric resonator. The oscillating piezoelectric resonator is attached to biomolecules, such as enzymes or antibodies, and can recognize target biomolecules with high selectivity. Giamblanco et al. made a piezoelectric biosensor to selectively monitor HBV, which validated detection of $10^{-15}$ mol cm$^{-3}$ HBV when probe density was around $4 \times 10^{10}$ molecules cm$^{-2}$. Moreover, this method is a single-step method without using any labels demonstrating its promising application in the development of portable POC biosensors for the diagnosis of HBV (Figure 11d).

DNA microarray biosensors have been used to understand the interaction between DNA or biomolecules, such as antibodies. Microarray is a solid substrate, such as a glass slide or a silicon wafer on which DNA or oligonucleotides either from pathogen or host are imprinted or attached. Further, fluorescent-labeled
cDNA is used to specifically detect the counterpart of the DNA, which has been bound on the substrate. Based on this principle, a library of DNA samples is imprinted on a single chip, and their genetic interactions can be screened (Figure 12a).

A similar strategy has been introduced for protein and glycan microarrays to detect and analyze their counterparts' interactions. For instance, several antibodies were imprinted on the surface of a silicon wafer/glass slide and specific virus strains were passed/incubated over the imprinted antibody. Then, a secondary fluorescent probe was used to detect the binding nature of the target virus antigen. Therefore, microarray biosensing is a promising technology for diagnosing and monitoring infectious diseases, such as HIV and influenza. Gallerano et al. developed an HIV microarray chip containing a set of proteins covering HIV-1 clade C's proteome. They demonstrated specific detection of immunoglobulin classes (IgM, IgA, IgG, and IgE) and subclasses (IgG1-4) against HIV antigens and peptides. Additionally, the antibodies detected the antigens of HIV in a femtogram range, indicating high microarray technology's sensitivity. In another study, a DNA microarray was designed to detect two influenza B lineages. Non-labeled viral RNA of 62 influenza B virus samples were hybridized to DNA capture sequences on a microarray in a sandwich process. A specific 5'-Quasar Q570 dye-containing DNA label sequence was introduced to detect RNA of the influenza B virus. The assay results showed that the detection of viral RNA was achieved in 8 h with 97% sensitivity and 100% specificity. Long et al. introduced a universal microarray containing 108 spots in nine rows for the detection of SARS-CoV (Figure 12b). The result is shown in Figure 12c, while cDNA samples of healthy persons did not show any signal.

In a recent study, Jiang et al. designed a microarray of SARS-CoV-2 proteome to identify specific IgM and IgG responses of COVID-19. 18 proteins from SARS-CoV-2 were printed on a microarray slide, then convalescent serum from COVID-19 patients was incubated on slides. The results revealed that IgG and IgM antibodies in the serum specifically bound to the SARS-CoV-2 proteins indicating the importance of microarray biosensors in screening viral protein detection.

6. Fabrication of Micro and Nanoscale Devices

COVID-19 spreading across the world showed how essential it is to develop easy-to-use and low-cost devices to rapidly recognize viral diseases. Conventional techniques, such as micromachining, computer numerical control milling, and CO2 laser cutting have widely been used to fabricate low-cost microfluidic...
platforms for rapid virus diagnosis.\[200,226\] In these techniques, the desired pattern is prepared using computer-aided design software and imported to a manufacturing process machine. Hot embossing is another technique for the fabrication of microstructures to form microfluidic biosensing devices. It has some advantages over conventional methods (e.g., lithography and etching) in productivity, resolution, and cost.\[227\] This technique includes heating a mold and polymer sheet to glass transition temperature \(T_g\) of the polymer, pressing a mold into the polymer to emboss the mold pattern into the polymer at the constant \(T_g\) temperature, and reducing the temperature for demolding.\[228\]

Soft lithography is another useful technique to create micro and nanofabrication on surfaces using elastomeric materials. Preparing microfluidic devices using soft lithography technique is low-cost and straightforward. A commonly used technique for fabricating microfluidic devices includes casting PDMS on a SU-8 mold, curing and peeling away the PDMS, and then sealing it against a glass slide.\[229\] Soft lithography techniques have also enabled the creation of micropatterns on soft substrates. The other examples of soft lithography techniques for microfluidic applications include replica molding, micro-transfer molding, and microcontact printing. Selective biomimetic surfaces can be fabricated for virus detection using the surface imprinting technique. To this end, a monolayer of biomacromolecules (i.e., virus particles) is deposited on a substrate to be used as a stamp.\[230\] The prepared stamp is then pressed onto a prepolymer substrate coated on a quartz crystal microbalance (QCM) electrode, and the prepolymer is left to polymerize under mild conditions. Then, the template is washed away to obtain a surface with imprinted biomacromolecules. Since the structural details of the biomacromolecules are transferred to the surface of the polymeric layer, a selective selection of target analytes can be achieved using this technique. A schematic of soft lithography is presented in Figure 13a.

Two and 3D printings are emerging technologies for the fabrication of complex POC virus diagnostic devices. In addition to direct extrusion 3D printing of microfluidic devices and biosensors, 3D printing of thermoplastic filaments using fused deposition modeling can also be done, especially for housing elements and electronics involving in a sensor platform.\[231,232\] Besides, inkjet printing of conductive ink patterns (e.g., AgNPs and graphene flakes) as electrode arrays, or non-conductive materials (e.g., polyimide) dielectric layers, on top of a flexible substrate has abundantly been reported for fabrication of biosensors.\[233,234\] Combining 3D printing with other conventional manufacturing methods, such as machining, milling, and lithography enables the fabrication of complex platforms including a broader range of materials and designs.\[231,232,235\]

Screen printing is a facile, scalable, and low-cost technique for the fabrication of biosensors.\[236\] In this technique, a stencil mask is laser-cut into the desired geometry, then the ink is spread over the desired substrate using a blade, while the stencil blocks the ink in some regions as represented in Figure 13b. Conductive materials can be screen printed on various substrates and used as electrodes for biosensing applications. Printing extra layers of graphene oxide with large oxygen-rich functional groups can augment their reactivity with biomolecules.\[237,238\] Besides, thin-film electrodes can be fabricated through other approaches, such as vapor deposition of metallic nanoparticles (e.g., AuNPs) called the sputter deposition process. In this process, target atoms are ejected onto a substrate to form a thin film of the material.\[239,240\]

The fabrication of paper-based microfluidic chips and biosensors is a simple and low-cost process. The use of paper is associated with several advantages, such as low-cost, biodegradability, and self-driven fluid flow due to capillary forces; however, there are also some limitations associated with use of paper-based techniques, such as opaqueness, poor flow control, and limited mechanical stability.\[241\] To tackle these problems, hybrid platforms comprising papers and polymeric materials have been developed to improve the performance of current paper-based devices.\[119,241\] Depending on the fabrication strategy and application, various types of papers (e.g., cellulose and nitrocellulose papers) can be selected as the building materials. Besides, commercially available surface-modified papers mixed with inorganic fillers are smoother and can act as an ion-exchange substrate, enabling further surface chemical modifications.

Nitrocellulose membranes with a specific pore size and smooth surfaces allow for a stable and repeatable fluid flow in paper-based microfluidic platforms.\[241\] Paper-based microfluidic platforms can be fabricated through a multi-step photolithography technique. In the first step, the paper is impregnated with a photo-resistant material, and then covered with a photomask and cured with UV light. Then, the extra unexposed photoresist is removed, while the hydrophobic barriers are generated within the paper (Figure 13c).\[242,243\] The hydrophobic barriers can also be created by wax printing on the paper sheet. In this method, the wax is directly 3D printed and penetrates into the paper sheet to create hydrophobic patterns within the hydrophilic paper sheet (Figure 13d).\[175,244\] An alternative approach for generating microfluidic channels is laser cutting and assembling layers of paper, polymethyl-methacrylate sheets, and double-sided tape adhesive.

Xurography is a print-and-peel technique for the fabrication of microfluidic platforms. A computer-controlled cutting plotter is used to cut thin-film polymers or papers for creating molds. Since there is no need for a cleanroom, xurography is considered to be a low-cost fabrication process compared to photolithography.\[78\] Using xurography, two and 3D microfluidic channels can be fabricated with features as small as 20 \(\mu\)m. For manufacturing tape-based molds, a tape is adhered to a backing substrate (e.g., a glass slide) and is cut based on a predefined pattern using a cutting plotter.\[245\] Then, the excess tape is peeled off from the glass, and a mixture of PDMS with curing agent is cast into the mold. After PDMS was cured, the patterned PDMS is separated from the mold. The fabrication steps are represented in Figure 12e. Thicker 3D patterns on PDMS can be obtained by adding extra layers of the tape on top of each other.

A new generation of electrochemical devices was developed during the past decade using advanced nanobiosensing technologies, such as a printed circuit board (PCB). Lab-on-PCB has enabled the integration of microfluidics, biosensors, and electronics in a single platform for complex sample analysis (Figure 13f).\[246–248\] Recently, PCB-based electrochemical devices were developed to rapidly detect COVID-19 antigens in saliva.
samples.\textsuperscript{[250]} Besides, micro-electro-mechanical systems have also enabled the integration of tiny electrical and mechanical components. MEMS technologies mostly use silicon and glass substrates and lithography techniques to fabricate microfluidic and lab-on-chip devices.\textsuperscript{[78]}

### 7. Commercialization

High-quality assays with sensitivity and specificity of higher than 95% should be used to diagnose viral infections. Major challenges in the development of diagnostic devices are a) detecting a small value of RNA virus to decrease false negatives, b) differentiating positive signal between various pathogens to reduce the number of false positives, and c) exhibiting an extensive capacity for performing rapid and correct tests for a significant number of patients, while eluding false negatives and false positives. Current diagnosis devices mainly use serological assays, which are based on either the exhibition of IgM antibodies or a notable enhancement in the level of IgG antibodies. However, serological assays have restricted sensitivity at primary steps when the host has not yet created particular antibodies. Studies on SARS-CoV-2 reported that the generation of IgM and IgG starts after the first week from infection of the virus, and they are detectable after 14 d.\textsuperscript{[252]}

Recently, major efforts have been conducted worldwide by pharmaceutical companies, commercial vaccine manufacturers, and laboratories at universities to accelerate the

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**Figure 13.** Fabrication techniques for micro and nanoscale devices. a) Soft lithography for surface imprinting of biomacromolecules. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License.\textsuperscript{[230]} Copyright 2017, MDPI Open Access Journals. b) Screen printing of carbon electrodes. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License.\textsuperscript{[238]} Copyright 2019, Elsevier c) Photolithography for the fabrication of paper-based microfluidic channels. Reproduced with permission.\textsuperscript{[242]} Copyright 2015, Elsevier. d) Wax printing for patterning wax barriers on a paper sheet. Reproduced with permission.\textsuperscript{[235]} Copyright 2019, Elsevier. e) Xurography for the fabrication of microfluidic channels. Reproduced with permission.\textsuperscript{[245]} Copyright 2019, Elsevier. f) Lab-on-PCB. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License.\textsuperscript{[249]} Copyright 2015, MDPI Open Access Journals.
discovery of therapeutic solutions against viral infections using new technologies.\textsuperscript{233} The development of microfluidic devices and POC of NAs can improve health monitoring and prevent viral infectious diseases. Commercialized POC devices should perform sample-in-answer-out DNA/RNA analysis and provide rapid and reliable test results for physicians and patients.\textsuperscript{234} NAs amplification requires equipped laboratories, experienced personnel, and laboratory operating expenses. New technologies have provided automation and miniaturization of steps, including the preparation of virus genome, amplicon detection, and amplification of NAs. These systems have made viral infection analysis more widely affordable.\textsuperscript{116}

There are commercialized systems and technologies based on antibody detection for the diagnosis of viral infection. Rapid technologies, such as POC diagnostic devices for detecting various types of viruses and viral antigens with a user-friendly solution should be commercialized.\textsuperscript{78} Several commercial devices for rapid viral detection, such as the Cobas influenza A/B & respiratory syncytial virus (RSV), GeneXpert Mycobacterium tuberculosis/resistance to rifampicin, Simplexa flu A/B, RSV direct, and Veredus Laboratories Pte. Ltd. system are shown in Figure 14.\textsuperscript{78} The Veredus Laboratories Pte. Ltd. made a POC for viral detection.\textsuperscript{255} The POC device contains two chambers with triangular profiles in a silicon chip. A chip is formed from a heater and sensor of temperature on a PCB.\textsuperscript{256} For conducting the test, a sample is loaded inside the chambers by a vacuum and the RT-PCR to amplify NAs. Fluorescence detection using a fluorescence camera system was utilized to detect the NAs. The RIDAQUICK is another rapid test based on a lateral-flow assay, which detects viral antigens using a cassette or a dipstick.\textsuperscript{257} It is used for the detection of adenovirus, norovirus, and rotavirus in a few minutes. Another commercialized kit, Binaxnow influenza A & B test kit, was developed for the qualitative detection of influenza virus type A and type B.\textsuperscript{258} Furthermore, several commercially available tests for rapid detection of HIV, including HIV 1/2/O rapid test device (assistance based on need (ABON)), Multisport HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories), Determine HIV 1/2 (Alere), OraQuick Rapid HIV-1/2 Antibody Test (OraSure Technologies), and DPP HIV 1/2 (Chembio) have been developed in POC setting to detect and differentiate between antibodies. Flow cytometry enzyme immunoassays and quantitative RT-PCR have been used for quantification of CD4 + T-lymphocytes and HIV. However, these methods are restricted by long turn-around time, the requirement for complex equipment, good-trained operators, and high costs. Therefore, POC diagnostics devices for detecting viral infection should be precise, low-cost, and user-friendly.\textsuperscript{259} Different methods, such as electromagnetic methods, nanoscaled electrochemical detection, surface plasmon resonance, nanomaterial-amplified optical tests, and surface-enhanced Raman Spectroscopy for rapid detection of HIV infection have been commercialized. However, physicians require a connected POC diagnostic device with smartphones for in situ and real-time detection of the virus.\textsuperscript{260}

Micro and nanotechniques including microfluidics play essential roles in commercializing POC devices. Most commercial systems are either DNA primer or antibody, and they are suitable to diagnose a broader range of diseases. However, some challenges, such as financing to produce viral infection devices, show the importance of governmental financial support for companies that make POC diagnostics devices.\textsuperscript{265} NanoSign HBs POC testing strip is another commercially available device for the diagnosis of HBV. This paper-based POC testing strip has a specific capability to meet the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) criteria for diagnosis applications in developing nations. However, this POC nanodiagnostic system is limited with low accuracy and detecting sensitivity. For instance, it was used for screening serum samples from 297 HBV infected patients, and false-negative results were usually observed.\textsuperscript{138} A list of microfluidic devices for the detection of COVID-19, their features, and technologies are presented in Table 3. The commercial kits for diagnosis of COVID-19 employ different lab-on-chip techniques to target fragments of N, S, ORFlab, and ORF8 genes of the SARS-CoV-2 virus. BioFire COVID-19 Test, Abbott NOW COVID-19 Test, NxTAG CoV Extended Panel, Hologic SARS-CoV-2 Assay, NeuMoDx SARS-CoV-2 Assay, BD MAX System, QIAstat-Dx Respiratory SARS-CoV-2 Panel, and ePlex SARS-CoV-2 Test are the commercial kits that are approved by Food and Drug Administration for diagnosis of COVID-19. In conclusion, microfluidic devices and micro/nanotechnology have a considerable role in viral diagnosis. These devices enhance operational simplicity, the limit of detection, and the cost-effectiveness of virus detection.

8. Conclusion and Future Prospects

The diagnosis of viruses is usually performed based on the detection of either virus particles or NAs. The conventional viral diagnosis for the diagnosis of viral particles or NAs is often accompanied by low sensitivity, long processing time, high cost, and complexity, which require skilled and trained users in laboratories to sample and process the viral particles from suspected
Technologies. We first described the devices, including MN patches and diagnosing viral diseases focusing on micro and nanotechnologies. We employed for sensing biomolecules. Additionally, the functionalized micro and nanoparticles can be integrated with these systems to enhance the capturing of target biomolecules and provide optical or non-optical properties on user-friendly and low-cost microfluidic channels with a high surface-to-volume ratio, which require a low volume of sample, thus reducing the consumption of samples and expensive reagents. Reducing the necessity of skilled workers and accelerate the proportion rate during pandemic events as compared to conventional methods, such as collection of exudates from airways and buccal cavity. The majority of microchip devices are based on user-friendly and low-cost microfluidic channels with a high surface-to-volume ratio, which require a low volume of sample, thus reducing the consumption of samples and expensive reagents. Additionally, the functionalized micro and nanoparticles can be integrated with these systems to enhance the capturing of target biomolecules and provide optical or non-optical properties employed for sensing biomolecules.

In this review, we summarized the advances in sampling and diagnosing viral diseases focusing on micro and nanotechnologies. We first described the devices, including MN patches and exhaled instruments, introduced to take samples from blood, urine, saliva, and breath for viral disease diagnosis. We also explained microfluidic techniques developed for sample processing to separate and extract the target biomarker and exclude unwanted biomolecules. Micro and nanotechnologies for enrichment, amplification, enhancement of capturing, and sensing of target biomarkers are described. Finally, the fabrication of microchips and microfluidic systems and the commercialized viral diagnosis methods are summarized.

The progress in micro and nanotechnologies in viral diagnosis resulted in the fabrication of commercialized microfluidic-based devices to diagnose different global viral diseases. Integration of other diagnosis steps, including sampling, sample processing, amplification, and sensing in a single, user-friendly, home setting, the chip-based device will re-shape the viral diseases’ diagnosis toward the self-diagnosed fully automated POC settings soon. Additionally, mobile health care is an important area that is attracting attention in developing devices for remote monitoring of health and diagnosing viral diseases in the regions with a poorly accessible health system. The integration of microfluidic systems with smartphone cameras with

Table 3. List of microfluidic devices for the detection of COVID-19 and their producers.

| Technology                  | Technical features                                                                 | Name of device                  | Company                        | Website                   |
|-----------------------------|------------------------------------------------------------------------------------|---------------------------------|-------------------------------|---------------------------|
| NAs Lab-in-Cartridge        | Lysis of samples by agitation and purifying by magnetic beads, freeze-drying reagents inside the device, detection by endpoint melting curve, 45 min test time | BioFire COVID-19 Test           | BioFire Defense, LLC          | biofiredefense.com        |
| NAs Lab-in-Box              | Lysis of samples by buffer, lyophilizing reagents, fluorescence detection, 13 min test time, predicted inclusivity of 100% | Abbott launching ID NOW COVID-19 Test | Abbott Laboratories         | abbot.com                 |
| NAs Lab-in-Plate            | Employing bioMérieux, easyMAG and EMAG systems for extraction of NAs, fluorescence detection, 4 h test time, works with MACPIX instrument and ORF1ab | Luminex, NxTAG CoV extended panel | Luminex Corporation          | luminexcorp.com           |
| NAs Lab-in-Tube             | Lysis of samples in a specimen transport tube and purifying by magnetic separation, fluorescence detection, 24 h test time for 1150 tests, can be used for coronavirus and other respiratory viruses | Hologic SARS-CoV-2 assay        | Hologic, Inc                 | hologic.com               |
| NAs Lab-on-Chip             | Lysis of samples by buffer in extraction plate wells containing coated paramagnetic beads and heating the plate, fluorescence detection, 1 h test time, works with NeuMoD 288 or NeuMoDx 96 Molecular Systems, lyophilization is required | NeuMoDx SARS-CoV-2 Assay        | NeuMoDx Molecular, Inc       | neuromdx.com              |
| NAs Lab-on-a-Chip (microfluidic PCR cartridge) | Extraction of NAs using BD MAX Exk TNA-3 kit, fluorescence detection, works with fully automated BD MAXTM system, Targeting N1 & N2 genes | BioGX SARS-CoV-2 Reagents (BD MAX System) | BioGX                | biogx.com                 |
| NAs Lab-in-Cartridge        | Lysis of samples using high-speed rotor and purifying by a membrane, fluorescence detection, 1 hour test time, works with QIAstat-Dx Analyzer system, fully automated assay in the cartridge, ability to differentiate coronavirus from 20 other respiratory infections | QIAstat-Dx respiratory SARS-CoV-2 panel | QIAstat-Dx                | qiastat-dx.com            |
| NAs Lab-on-Chip             | Extraction and purification of NAs through magnetic solid-phase extraction on digital microfluidic chip, electrochemical signal detection, 90 min test time, works with ePlex system, fully automated assay in the cartridge | ePlex SARS-CoV-2 Test (ePlex System) | GenMark-Dx                  | genmarkdx.com             |
high resolution enables the sensitive detection of optical signals and sending the results to the healthcare authorities to directly monitor the disease outbreaks. The products can also be evaluated and analyzed using user-friendly software, which can be easily used on smartphones.

In contrast to the recent advances in this area, there are significant challenges in developing these devices. For instance, achieving the desired sensitivity from a small volume of samples in chip-based devices is a critical issue for viral diseases like HIV, which usually have a low viral load. These samples typically require a preconcentration step before loading to the device. As a result, it is still necessary to collect a high volume of representatives from the patients and concentrate the sample by reducing its volume. Additionally, in contrast to the extensive research in the development of microfluidic systems and nanoparticles for sample processing and virus detection, the application of MN patches and exhale devices with high capturing efficiency for viral sampling is still in its early stages and needs further investigation. Also, the integration of MN patches and exhale devices with microfluidic systems for sampling, amplification, and sensing in a single integrated device has not been investigated yet. Moreover, the development of micro and nanotechnology for precise and rapid measurement of the viral load from a drop of blood in a single-use device is a significant challenge for the diagnosis of viral diseases. Although micro and nanotechnologies enabled the development of commercial microfluidic devices for HIV diagnosis using CD4+ T cell counting from blood droplets, viral diagnosis through viral loading measurement is limited by low sensitivity.

Our experience in the COVID-19 pandemic demonstrated the critical need to overcome the challenges in developing reliable, fast, and cost-efficient POC diagnosis of viruses. In addition to the technical issues, transferring advanced research to the industry, scaling up and manufacturing costs, and necessary investments to commercialize the techniques are significant challenges that should be seriously considered in the future. To overcome the worldwide pandemics, such as COVID-19, the companies need to raise urgent funds, which show the critical role of governmental, non-governmental, and philanthropic foundations to invest in this area. Additionally, as the development of diagnosis devices requires fundamental knowledge in various areas, the development of interdisciplinary courses in educational institutions should be encouraged in the future. Moreover, clinical validations and optimization of clinical pathways are necessary for these developed technologies to be successfully translated from research to clinical applications.

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Conflict of Interest
The authors declare no conflict of interest.

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