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Molecularly Imprinted Polymers: Promising Tool for the Human Virus Detection

Muhammad Shahzeb Khan[1]*, Muhammad Ibrar Asif[1], Amina Khatoon[1], Shafia Arshad[2], Shagufta Usman[3] and Iqra Karim[4]

[1]*Sulaiman Bin Abdullah Aba Al-Khail Centre for Interdisciplinary Research in Basic Sciences, International Islamic University Islamabad, Pakistan.*

[2]*Faculty of Medicine and Allied Health Sciences, Islamia University Bahawalpur, Pakistan.*

[3]*Faculty of Pharmaceutical Sciences, Riphah International University Islamabad, Lahore Campus, Pakistan.*

[4]*Department of Chemistry, COMSATS University Islamabad, Lahore Campus, Pakistan.*

**Corresponding Author Email: mshahzebkhan006@gmail.com*

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Abstract

Molecular imprinting is an attractive research area for synthesizing unique functional polymers with high selectivity due to template oriented active sites. Molecularly imprinted polymers (MIPs) have a wide range of applications in chemical and biological sensing, drug delivery, and solid-phase extraction owing to mechanical stability, reversibility, reproducibility, and cross-validity. MIPs are compatible with natural antibodies and are being used as antibody mimics/receptors in the biomedical field. Today, viral detection is the most popular research area due to emerging viral diseases with genetic variability and drug resistance. Therefore, there is a need to control viral infections by discriminative recognition of the viral pathogens. This review summarizes the literature on the detection of human viruses by using MIPs.

Keywords. Molecularly imprinted polymers, Human viruses, Viral detection

Introduction

Viral infections are infecting the world due to their emergence and rapid spread from one region to another. Despite many efforts to control viral infection, it is still a major threat to the human population because of rapid spread and genetic variability [1-5]. Every year, 17 million deaths are caused due to viral infectious diseases [6]. It is challenging to control the viral disease because of the genetic variability, multi-resistant pathogen, absurd use of antibiotics, and rapid transmission [7, 8]. Timely and selective diagnosis following targeted vaccination can be useful to control viral infectious diseases. Some diagnostic techniques that are under practice for the laboratory diagnosis of the viral infectious disease are polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), chest CT, microscopy, and culture [9-14]. Several disadvantages are associated with these techniques, including complexity, time-consuming, non-economical, non-portable, and required sample preparation [15]. There are also some biosensors and bioassays that are present to detect viral pathogens, but there is a need for cheap, portable, and reusable biosensors for the sensitive and selective detection of various viral pathogens [16, 17]. Recently, molecularly imprinted polymers (MIPs) are being prepared by the utilization of molecular imprinting technology, which is compatible

with natural antibodies owing to their unique characteristics. MIPs have specific imprinted cavities that bind only specific template molecules of interest, like a lock and key model [18]. Functional monomers interact with the templates, and a pre-polymerized complex is formed. The polymer matrix is obtained by adding porogenic solvents, cross-linker, and initiator. The polymer matrix is washed with an appropriate solvent to eliminate the template molecule to generate a size identical imprint of the target molecule [19, 20]. MIPs are being used in the sensing and separation ranging from macro to micromolecules because of their excellent features. MIPs not only bind the template molecule but also possess tolerance to mechanical stress, elevated temperature, pH, and acid-base environments [21]. MIPs are appropriate for various kinds of applications such as recognition receptors for chemical biosensors, mock elements for drug analysis, chromatographic separation media, solid-phase extraction, plastic antibodies, and drug delivery Imprints of a vast range of the templates have been generated such as serotonin, urea, salbutamol, glucose, gallic acid, hydrazone, atrazine, cefalexin, testosterone, bilirubin, prostate cancer cell streaks, paclitaxel, uric acid, valganciclovir, various metals, and volatile organic compounds [20, 22-27]. Figure 1 represent the schematic illustrations for the synthesis of MIPs.

Viruses can mutate and produce new variants, which cause reinfection and fast transmission of the virus. Therefore, a fast and selective diagnosis of the viral infection is needed to control and treat the disease. MIPs are the artificial receptors for the detection of emerging virus infections compatible with natural antibodies due to their high sensitivity, selectivity, portability, low cost [28, 29]. In this review, we shall focus on the surrounding literature related to the recognition of human viral pathogens by utilizing the MIPs.

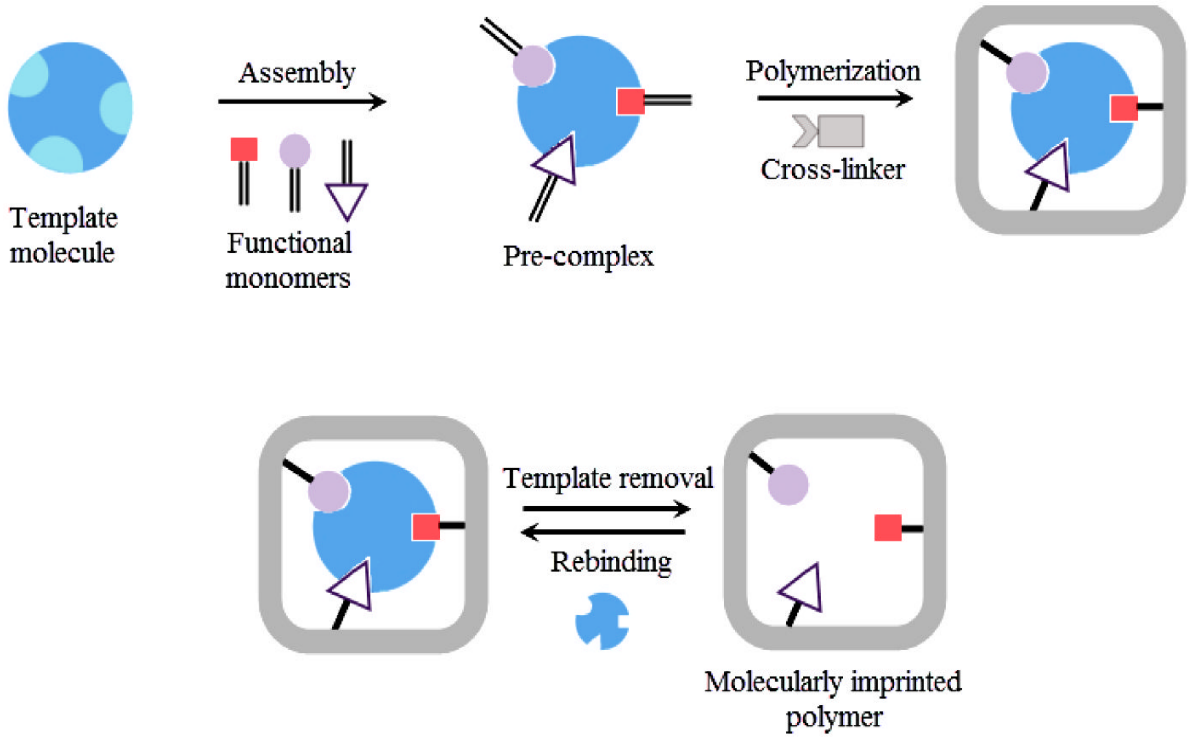

Figure 1. **Fabrication mechanism of MIPs [18]**

Dengue Virus

Dengue is a mosquito-borne disease that affects the human population worldwide. Dengue virus has become a major health threat to the public because of its viral genetic variation and transmission [30]. According to WHO, 100-400 million people get infected annually by the dengue virus [31]. There are severe clinical problems caused due to non-specific demonstration leading to misdiagnosis of the infection. The development of the dengue disease vaccine is still in progress. Therefore, there is a need for fast disease diagnoses and patient management [32]. Sukjee *et al*. reported the imprinted polymer composite to recognize the dengue virus. Hummer's method was used to synthesize Graphene oxide (GO). The polymer was generated by mixing the methyl methacrylate (MMA), methacrylic acid (MAA), acrylamide (AAM), and n-vinylpyrrolidone (NVP). The GO sheets and polymer were assorted to obtain a polymer composite with enhanced sensitivity. The polymer/GO composite was spin-coated on the gold electrode; the dengue virus and BPA template were plunged on the thin polymer film. The polymerization further proceeded under UV light for 2 hours at 55 ^{o}C. Non imprinted polymer (NIP) based sensor was also synthesized to compare the response. The response of the electrochemical sensor was linear towards BPA concentration. The sensitivity response of the imprinted polymer-based sensor was high than the non-imprinted based sensor with the lowest detection limit of 0.1 pM due to template oriented cavities within the polymer matrix [33]. Table 1 summarizes the MIP based sensors for the detection of dengue virus.

Rabia *et al*. reported MIP based impedance sensor for the detection of the dengue virus. Firstly, a mixture of dopamine hydrochloride and NS1 protein was formed, and 20 µL of the mixture was placed on polysulfone-modified Screen-printed carbon electrodes (SPCEs), and polymerization continued. The electrode (SPCE/PS/NS1/DA) was treated with buffer after the polymerization. MIP modified electrodes were treated at various concentrations of NS1 protein to evaluate the sensitivity and selectivity response. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used to measure the electrochemical response of MIP based sensor. The sensor exhibited a linear impedimetric response from 1 to 200 ng/mL with the LOD of 0.3 ng/mL. Real human serum samples were also tested with the developed sensor to determine the NS1, and adequate recoveries altering from 95 to 97.14% were attained [34]. Lieberzeit *et al*. reported a MIP-based QCM sensor for the detection of the dengue virus. The polymer was synthesized by using MAA and NVP monomers and cross-linker EGDMA. Dengue virus imprint was generated into the polymer matrix through molecular imprinted technique and NIP was also synthesized to compare the response. MIP response was 3 times higher than the NIP response with linearity curve [35].

***Table 1*. MIPs for Dengue virus detection.**

Virus	Template	Functional Monomer	Porogenic solvent	Transducer	Detection limit	Ref.
Dengue virus	BPA with dengue	MMA, MAA, NVP	-	Electrochemical	0.1 pM	[33]
Dengue virus	Dengue NS1 protein	Dopamine	DMF and THF	Electrochemical	0.3 ng/mL	[34]

Influenza Virus

Influenza, commonly named flu, is caused by viruses, belongs to the orthomyxoviridae family. Influenza viruses comprise a single-stranded negative RNA genome that encodes eleven proteins [13, 36]. The Orthomyxoviridae family has four types of influenza virus genera: A, B, C, and D. This classification is based on the matrix and internal glycoprotein nucleoprotein. A, B, and C genera generally infect human beings and cause significant illness and death per annum, whereas D type only infects cattle. Type A virus is among the most concerning type of viruses because it is responsible for severe seasonal epidemics [37]. Nearly 80% of influenza virus surface contains neuraminidase (NA) and glycoproteins hemagglutinin (HA) [38, 39].

Every year both A and B type causes deaths of nearly 30,000 to 50,000 persons while 200,000 people are hospitalized. Influenza is a leading cause of death after AIDS because approximately 5 – 15% of global population deaths occur per annum estimated by WHO. H1N1 and H3N2 subtypes also caused four major pandemics: Spanish flu, Asian flu, Hong Kong flu, and H1N1 pandemic in 1918, 1958, 1968, and 2009, respectively [13, 41]. MIPs are being used as selective bio mimics for the detection of the influenza virus. Five different MIPs QCM based sensors were generated by using five common strains of influenza A (H5N1, H5N3, H1N1, H1N3, and H6N1). Wangchareansak *et al.* used various monomers having concentrations as 13.0 mg acrylamide, 10.6 mg methacrylic acid, 6 mg methylmethacrylate, and 6.3 mg N-vinylpyrrolidone were mixed into a cross-linker which is 48 mg N, N-(1,2-dihydroxy ethylene) bisacrylamide. The solvent used in this method was 300 mL DMSO, and 1 mg AIBN was used as an initiator. After that ability of MIP to detect template viruses was measured by using QCM [38]. QCM was used to measure the ability of molecularly imprinted polymer to absorb H5N1. These MIPs were used to identify inhibitors that can bind to the host receptors and obstruct virus function by making conformational changes [42]. Figure 2 shows the gravimetric detection of influenzas viruses.

MIPs as plastic antibodies were designed by Sangma *et al.* and used as copolymer systems for the detection of H5N1. This copolymer system is comprised of four types of monomers, including AAM, MAA, MMA, and vinylpyrrolidone [43]. Table 2 summarizes the MIP based sensors for the detection of the influenza virus.

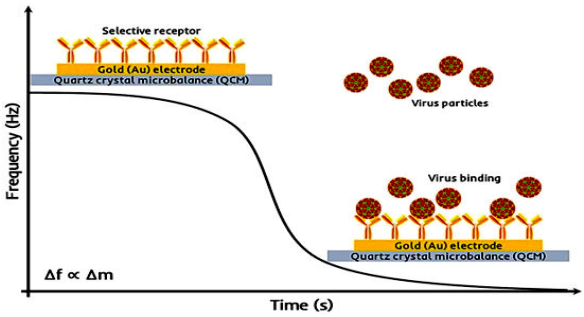

***Figure 2*. QCM based detection of influenzas viruses [40]**

***Table 2*. MIPs for the detection of influenza.**

Virus	Template	Functional monomer	Crosslinker	Initiator	Porogenic solvent	Transducer	Ref
Influenza	H5N3, H1N3, H6N1	AAM, MAA, MMA, Vinylpyrrolidone	DHEBA	AIBN	(DMSO)	QCM	[38]
Influenza	H5N1	AAM, MAA, MMA, Vinylpyrrolidone	AIBN, DHEBA	AIBN	DMSO	QCM	[43]
Influenza	H1N1	AAM, MAA, MMA, Vinylpyrrolidone	EGDMA, DHEBA	AIBN	DMSO	QCM	[44]

New granular MIP was synthesized by Sukjee *et al*. through precipitation polymerization using the influenza A virus as a template. The selective binding of influenza virus to molecularly imprinted polymer was measured after that, it was compared with other viruses. An agglutination test was done to check the affinity of the virus towards MIP. The MIP formed using the H1N1 template displayed specific reactivity, while the MIP formed using H5N1 and H3N2 templates exhibited cross-reactivity [44].

Hepatitis

It is a cluster of infectious diseases, and it is caused by hepatitis A, B, C, and D. Same disorder caused by these four types is liver function damage. These four types of viruses can also infect each other and live together in inpatient production with great complications. Hepatitis A virus and hepatitis B virus are the most common type [41].

Hepatitis A Virus

HAV is a chief public health problem, and significantly it is a human pathogen. HAV is spread by the fecal-oral route and by contact with the infected person HAV causes liver inflammation, and contaminated food is a common mode of Hepatitis A infection. The long-lasting liver infection causes a patient's death by sudden liver failure [45].

Upon clinical presentation, HAV cannot be distinguished from other hepatitis viruses due to structural similarities. Molecularly imprinted polymers have been proved very useful for HAV detection because of highly selective HAV detection. Another study showed that for precise identification and similar virus detection, molecular imprinting technology was a promising tool, while Quantum dots (QDs) were used to accomplish this goal. Green QDs@MIP and red QDs@MIP were produced by imprinting two different viruses on two different imprinting substrates. The hydrophilic group N-isopropyl acrylamide and zinc acrylate as a functional monomer were added to improve recognition ability to discriminate similar viruses. This strategy not only determined target viruses but also distinguished two viruses by mixing two different types of MIPs. Visual recognition by the naked eye was done with the help of fluorescence core selection, which is based upon large stokes shift and manageable emission wavelength of quantum dots [45]. For precise diagnosis, Yang *et al*. designed a new core-shell molecular imprinted nanoparticles having PDA capped with silicon dioxide SiO2@PDA. These novel core-shell HAV-imprinted nanoparticles showed some properties to the HAV template like hydrophilic, specific recognition, and biocompatibility. Resonance light scattering (RLS) and fluorescence spectrophotometer was used for specific recognition and detection, respectively. The light scattering of this novel core-shell depends upon HAV concentration. Greater HAV concentration greater will be light scattering and vice-versa. It also showed an excellent selectivity towards HAV-virus. This novel HAV-imprinted SiO2@PDA nanoparticles were successfully used for the detection of HAV in real serum. This method combined MIP and RLS. The advantages of this novel sensor were high selectivity, best sensitivity, low cost, simplicity, and excellent biocompatibility [46]. Another novel RLS sensor was designed on a silica surface using thermosensitive imprinted polymer and N- isopropyl acrylamide as a temperature-sensitive element. By using this sensor, selection detection of the HAV was demonstrated, and its recognition ability was regulated by temperature control. This sensor was highly selective, eco-friendly, short response time, simple, sensitive, and facile [47].

Hepatitis B virus

HBV causes about eight hundred thousand deaths annually because of cirrhosis and liver cancer. It is considered a major disease of humankind [48, 49]. HBV is highly contagious, spread by sexual activity, unsafe medical treatments, and transmitted from mother to child [48]. It is an extremely common infection in the world. About 15-40% of chronically infected persons will have hepatocellular carcinoma, cirrhosis, and liver failure, while 15-25% will die [48]. There are about eight major HBV genotypes in human beings. HBV genotype C is related to liver fibrosis and hepatocellular carcinoma [50]. HBV genome contains three thousand and twenty nucleotides long, and it consists of partially double-stranded DNA [51]. Similarly, the red MIP was synthesized by the same procedure (previously reported the synthesis of green MIP) by using the same chemicals with the same ratio except for the template, which is HBV in this case. The best amounts of EGDMA, zinc-acrylate, TEOS, and NIPAAm for R-MIP were 150 μL, 40 mg, 300 μL, and 20 mg, respectively [45].

Hepatitis C Virus

Patients having acute (HCV) infection develop chronic infection and a high risk of liver fibrosis progression. Advancement in alanine aminotransferase (ALT) is a significant predictor for enlargement and progression of liver fibrosis during chronic HCV infection. A novel sensor was designed for the detection of HCV core antigen by combining aptasensing, and MIP methodologies founded on electropolymerization of dopamine (DA) around the Apt [HCV core antigen] complex on MWCNTs-Chit modified GCE. It is a suitable method for HCV detection with high stability, low cost, best sensitivity, and short response time. CV, DPV, EIS are some electrochemical techniques that were used for measuring analytical performance [52].

Hepatitis E Virus

HEV is one of five known hepatitis viruses, and it was discovered after the hepatitis epidemic occurred in the early 1980s because this virus caused the epidemic. There are several genotypes, and it was revealed after molecular characterization. Infections in human beings are caused by HEV genotype 1 HEV1, HEV2, HEV3, and HEV4 [53]. HEV1 and HEV2 are prevalent in developing regions, while HEV3 and HEV4 are observed in developed regions. HEV mainly spreads through the food chain and by eating meat from infected animals. It causes liver failure, chronic hepatitis, and cirrhosis [53]. HEV3 is responsible for causing hepatitis E [53]. Various MIP based sensors for the Hepatitis virus are summarized in Table 3.

Table 3. MIPs for Hepatitis virus detection.

Disease	Template	Linear Range	LOD	Detection method	Ref
Hepatitis	HAV	0.04–6.0 nmol/L	8.6 pM	MIPs-RLS sensor	[46]
Hepatitis	HAV	0.3−95 nmol/L	3.4 pmol/L	Molecularly Imprinted Fluorescence Sensor	[45]
Hepatitis	HAV	-	1.1 pmol/L	Thermosensitive MIP RLS sensor	[47]
Hepatitis	HBV	0.5-90 nmol/L	5.3 pmol/L	Molecularly Imprinted Fluorescence Sensor	[45]
Hepatitis	HCV	5.0 fg/mL to 1.0 pg/mL	1.67 fg/mL	Aptamer MIP	[52]

Adenovirus

Adenoviruses are considered as non-enveloped, double-stranded DNA viruses linked with a wide range of medical disorders in human beings. Adenoviruses are responsible for gastrointestinal, self-limited respiratory conjunctival diseases [41, 54], hepatitis, pneumonia, and myocarditis in humans. While among children, five to seven percent of respiratory infections are caused by a human adenovirus (HADV) [41]. It can be transmitted via exposure to infected blood or tissue, inhalation of aerosolized droplets, fecal-oral spread, or direct conjunctival inoculation. The infection can be developed from two days to two weeks depending upon exposure to the virus and transmittance mechanism [54]. About eighty-eight different HADV types are recently assembled into seven human adenovirus species, including A to G [55].

Altintas *et al*. had designed Adenovirus specific-MIP nanoparticles immobilized on a surface plasmon resonance (SPR), and this was done with the help of glutaraldehyde coupling. MIP was designed using solid support, which is a glass bead in this case for adenovirus template followed by all constituents for molecularly imprinted polymer synthesis to obtain adenovirus-MIP nanoparticles with approximately 260nm size [41, 56]. Fabricated MIP based sensor exhibited excellent sensitivity within the concentration range of 0.02–20 pM and detection limit of 0.02 pM [56].

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) is a zoonotic virus transmitted by mosquitos from animals to humans and is the major cause of viral encephalitis, known as Japanese encephalitis (JE). The disease was first reported in Japan and was characterized in 1935 [57, 58]. Despite the effective vaccine development in 1941 and precautionary measures taken, the disease has emerged globally, especially in Southeast Asia and Australia. Annually 50,000 to 175,000 cases are reported leading to the death of more than 10,000 to 15,000 patients. Moreover, JE is considered the most frequent viral encephalitis with a high mortality rate (25 to 30%) and neuropsychiatric sequel disorders in 50% of recovered patients [59-61]. People of all ages are affected, but children are exposed more to JE due to their low immunity [62]. Several conventional methods like Enzyme-linked immunosorbent assays (ELISA), virus isolation, reverse transcriptase PCRs (RT-PCRs), and neutralization tests are used for the JEV virus. Still, scientists are making efforts to develop more sensitive, selective, and cost-effective methods for the detection of JEV. Among them, electrochemical and biosensors have emerged as the most sensitive and effective tools for virus detection [58, 63, 64]. Molecularly imprinted polymer-based viruses are being effectively used with several transducing systems to detect viral pathogens, specially JEV with high selectivity [65].

Simple MIPs are highly-selective, but they require complex signaling processes and instrumental confirmation of results. The introduction of fluorescent material into molecularly imprinted polymers can provide fluorescence detection of the specific target [66]. Liang *et al*. developed a fluorescence sensor for JEV detection on the surface of silica microspheres (SiMP). The surface of SiMP was modified with fluorescence dye (pyrene-1-carboxaldehyde). The fluorescence intensity was enhanced by using a fluorescence energy transfer mechanism in which the virus acted as a donor while the surface acted as an accepter. The intensity was found proportional to virus concentration with a maximum intensity at 960 pM. The observed detection limit was 9.6 pM with a relative

standard deviation of 1.99% [67]. Feng *et al.* developed a surface imprinted fluorescent MIP sensor on silica microspheres (SiMP) by using dansyl chloride (DNS-Cl) as a fluorescent material. After immobilization of DNS-Cl on the silica surface, the polymer was generated by mixing the template (JEV), monomer (3-Aminopropyl)triethoxysilane) and tetraethyl orthosilicate (TEOS) cross-linker with the silica microspheres, and the polymerization was performed. After template removal, the cavities were generated and the sensor was exposed to various template concentrations from 1.2 pmol·mL-1 to 960 pmol·mL-1. The sensor was able to detect JEV up to Pico molar concentrations with an equilibrium time of 55 minutes [68]. Using molecular orbital framework (MOF) as supporting material for MIP increases its selectivity and surface area, resulting in enhanced sensitivity and selectivity of the sensor [69]. Yang *et al.* used a fluorescence MIP sensor based on the molecular orbital framework for JEV detection. The polymer was formed with a free-radical polymerization method on silicon modified MOF surface using zinc acrylate as a monomer. Polyethylene glycol was used as a blocking agent for controlled polymerization and higher selectivity. The polymer responded to a wide range of virus concentrations 50 pmol L^{-1} to 1400 pmol L^{-1} with a very low response time of 20 min. The observed detection limit was 13 pmol L^{-1} under optimal experimental conditions [70]. Magnetic molecularly imprinted polymers (MMIPs) have provided a great advantage over conventional MIPs as they are more sensitive, have low operation time, and provide easy template removal [71]. Luo *et al.* fabricated a surface imprinted RLS magnetic sensor for JEV detection. The silicon-coated Fe_3O_4 microspheres were used as a substrate material, while the polymer was generated around JEV templates using Aminopropyl-triethoxysilane (APTES) as a monomer and polymerization process of Tetraethyl-orthosilicate. The magnetic removal of the template molecules created JEV specific cavities. The sensor exhibited excellent selectivity and sensitivity for the virus with a very low detection limit of 1.3 pM. The sensor had a very low response time and the absorption equilibrium was much lower (20 min) due to the combined advantages of RLS and magnetic MIPs [72]. The study reveals that the advances in imprinting mechanism and separation technologies the sensitivity and selectivity of fluorescence methods have been much approved. There is a great hope that soon, noninvasive hand-based sensing tools will be available for accurate on spot detection of JEV and associated encephalitis.

Table 4. MIPs for the detection of JEV.

Disease	Template	Linear Range	LOD	Detection method	Ref
Japanese encephalitis	JEV	20.6 pM to 980 pM	9.6 pM	Molecularly Imprinted Fluorescence Sensor	[67]
Japanese encephalitis	JEV	1.2 pmol/L to 960 pmol/L	0.4 pmol/L	Molecularly Imprinted Fluorescence Sensor	[68]
Japanese encephalitis	JEV	50 pmol/L to 1400 pmol/L	13 pmol/L	Magnetic Molecularly Imprinted Fluorescence Sensor	[70]
Japanese encephalitis	JEV	-	1.3 pmol/L	Resonance light scattering-Magnetic Molecularly Imprinted Fluorescence Sensor	[72]

Picornaviruses

Picornaviruses are a diverse group of non-enveloped positive-sense, single-stranded RNA viruses comprising more than 110 recognized species divided into 47 genera that may increase with time [73]. These viruses have diverse pathogenicity and affect many animal species and humans. They cause a variety of diseases such as acute or sometimes fatal paralysis, meningitis, myocarditis, poliomyelitis, and encephalitis [74]. They are also a source of foot and mouth infections and several clinically acute vesicular diseases in domestic animals such as ruminants and swine [75]. Quartz crystal microbalance (QCM) transducers have been frequently used to develop molecularly imprinted polymer-based sensors because of their highly sensitive response and structural variability [76]. A QCM based molecularly imprinted polymer sensor was developed by Jenik *et al*. for the detection of human rhinovirus (HRV) and foot and mouth disease virus (FMDV). Multilayers of polyurethane were generated and, virus imprinted cavities were generated by the stamp imprinting process. The sensor was exposed to various concentrations of virus template, and measurements were recorded. The results suggested that the template shows reversible binding that has a magnitude greater than the non-specific adsorptions. The sensor exhibited a net frequency change of -300 Hz when exposed to a viral suspension with template concentration of 100 µg/mL with a brilliant signal to noise ratio. The change in frequency was also observed between HRV and FMDV viruses. Different stereotypes of HRV such as HRV_1A, HRV_{14}, and HRV_{16} were also distinguishable due to different frequency changes by the virus molecules. Thus the proposed sensor proved to be highly sensitive and selective for HRV and FMDV detection and is a pathway to develop reliable sensors for on-spot detection of picornaviruses [77].

Conclusion

The molecular imprinting technique is an emerging technology for the selective detection of viruses through template-oriented cavities within the polymer matrix. MIPs has advantages over natural antibodies because they are selective, robust, economical, and stable. The world is still suffering from viral syndromes and the most recent pandemic of coronavirus. We also suggest fabricating a MIP-based coronavirus sensor using previous viral detection methods. This can be the most effective and selective sensor for the coronavirus disease diagnosis. Moreover, MIPs have been presented as a novel media for screening inhibitors for drug discovery that might be utilized in the battle against viral diseases. The complex of focal points achieved by the sub-atomic engraving innovation is foreseen to locate its home in the frontlines of future viral investigations.

Conflict of Interest

The authors declare that they have no conflict of interest.

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