Trends and Perspectives of Biosensors for Food and Environmental Virology

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Abstract Food and environmental virology has become a very important and interesting area of research because of food safety and public health concerns. During the last few decades, increasing foodborne diseases and environmental generated illnesses are considered to be highly challenging issues. Biosensor technology holds great promise for the healthcare market, and the security sector. Similar to clinical diagnostic tools, biosensors are being developed for the rapid, reliable, yet inexpensive identification and enumeration of pathogenic viruses which are adulterating environment, food and feed commodities. In this modern era, bio-and nano-technologies play a pivotal role in virological diagnostics of food industry, environmental and veterinary samples. This review covers the recent advances and future prospects of nanotechnology-based bioanalytical microsystems for food and environmental virology.

Keywords Biosensors · Nanotechnology · Food virology · Microsystems · Noroviruses

Introduction

Virus transmission via food and the environment route is now a serious problem of the utmost concern. It has been estimated that around 76 million people suffer foodborne illnesses yearly in USA, out of which 325,000 being hospitalized, of whom more than 5,000 die. Moreover, the annual direct-medical-expenses for these illnesses are estimated around US$ 5–6 billion (Mead et al. 1999). Virion-detection methods are of immense importance because of ensuring our wellbeing, investigating outbreaks and devising the preventive measures. Many challenges have to defeat to fabricate the sensors that could efficiently determine the identity and enormity of pathogens in a food or water sample in an easy, economical, and sensitive manner. Although, standard microbiological assays can furnish an eventual sensitivity and identify the occurrence of even a single cell in a provided food sample, however, this approach suffers from the long incubation steps. Also, their complex procedures mandate culture handling by only skilled technician. The bioanalytical microsystem which includes a microfluidic biosensor has been developed through nanotechnological techniques and is highly efficient for the pathogen detection. Recently, nanotechnology has become one of the most dynamic research disciplines in the fields of solid-state physics, chemistry, materials science, cytology, molecular biology, biotechnology, and other allied disciplines (Edvardsson et al. 2005; Niemeyer and Mirkin 2004). As nanobiotechnology advances, pathogen-detecting sensors or their components continue to be transforming into the miniature and the highly sensitive state. Since, the sensors are of nanoscale size; their sample from which the detection is being made is usually a microliter or even much small in size. In contrast to other detection systems, the biosensor detection strategies attribute several key features such as high sensitivity, fast response, robustness, low cost, miniaturization and simultaneous multianalyte detection.
Viruses Transmitted via Food and Environment

Viruses of at least a dozen families have been associated with foodborne illness, firing up various diseases which range from a mild respiratory disease to acute immunocompromised dehydrations or liver diseases leading to the hospitalization. Various food borne and clinically significant families of the viruses that cause illnesses in humans and other animals are summarized in Table 1. All the viruses set off a specific pattern of illness, as they have their own characteristic host range and the cell preferences. They can be transmitted through several modes including the respiratory route, faecal-oral route, sexual contact, zoonotic, and vectors mediated route (Cliver 2009). Although, all viruses including the human rotavirus (HRV), hepatitis E virus (HEV), astrovirus, aichi virus, sapovirus, enterovirus, coronaviruses, parvovirus and the adenovirus have a potential to cause foodborne illnesses, noroviruses (NoVs) and hepatitis A viruses (HAVs) have most frequently been involved in certain foodborne infections. Taking into account the infection symptoms, the viruses has been broadly classified into three major groups: (a) those which cause gastroenteritis (NoV, HRV, astroviruses, aichi virus adenoviruses and sapoviruses), (b) enterically transmitted hepatitis (caused by HAV and HEV, that pass to the liver, where they strike disease), and (c) which replicates in the human gut, but only causes illness if migrate to other organs such as the central nervous system (enterovirus). Normally, the viruses exist in the environment and acquire certain capability to endure routine food sterilization processes. Besides this, a few viruses may erratically be spread via food, even if their normal mode of transmission is different, as in case of severe acute respiratory syndrome (SARS)-causing coronavirus (SARS-CoV) and highly pathogenic avian influenza (HPAI) virus. Most of them are small spherical particles, with a single-

| Family      | Baltimore group (s) | Important species                          | Envelopment | Virion shape | Common diseases                                                                                     | Replication site |
|------------|---------------------|---------------------------------------------|-------------|-------------|--------------------------------------------------------------------------------------------------|------------------|
| Adenoviridae | dsDNA               | Adenovirus                                  | Non-enveloped | Icosahedral | Respiratory disease, gastroenteritis, conjunctivitis, cystitis and rash.                        | Nucleus          |
| Flaviviridae | +ssRNA              | Hepatitis C virus, yellow fever virus, dengue virus, West Nile virus, other Flaviviruses | Enveloped    | Icosahedral | Dengue fever, encephalitis, Yellow fever                                                       | Cytoplasrn       |
| Hepadnaviridae | dsDNA and ssDNA      | Hepatitis B virus                           | Enveloped    | Icosahedral | Hepatitis B                                                                                       | Nucleus          |
| Herpesviridae | dsDNA               | Herpes simplex, type 1, Herpes simplex, type 2, Varicella-zoster virus, Epstein-barr virus, Human cytomegalovirus, Human herpesvirus, type 7, Human herpesvirus, type 8 | Enveloped    | Icosahedral | Chickenpox and shingles, Infectious mononucleosis, lymphoma, post-transplant lymphoproliferative syndrome (PTLD), nasopharyngeal carcinoma, sixth disease | Nucleus          |
| Orthomyxoviridae | −ssRNA             | Influenza virus                             | Enveloped    | Spherical   | Influenza                                                                                         | Nucleus          |
| Papillomaviridae | dsDNA              | Papilloma virus                             | Non-enveloped | Icosahedral | Tumor                                                                                             | Nucleus          |
| Paramyxoviridae | −ssRNA             | Measles virus, Mumps virus, Parainfluenza virus, Respiratory syncytial virus, Human metapneumovirus | Enveloped    | Spherical   | Bronchiolitis and pneumonia                                                                       | Cytoplasrn       |
| Parvoviridae | ssDNA               | Human bocavirus, Parvovirus B19             | Enveloped    | Icosahedral | Slapped cheek disease, gastroenteritis                                                            | Nucleus          |
| Picornaviridae | +ssRNA             | Coxsackievirus, hepatitis A virus, poliovirus | Non-enveloped | Icosahedral | Polio, meningitis, hepatitis, and the common cold                                                  | Cytoplasrn       |
| Retroviridae | +ssRNA              | Human immunodeficiency virus (HIV)          | Enveloped    | Spherical   | AIDS, tumor                                                                                       | Nucleus          |
| Rhabdoviridae | −ssRNA              | Rabies virus                                | Enveloped    | Helical, bullet shaped | Rabies                                                                                           | Cytoplasrn       |
| Togaviridae  | +ssRNA              | Rubella virus                               | Enveloped    | Icosahedral | Rubella, sindbis, mayaro, chikungunya                                                             | Cytoplasrn       |
stranded positive-sense RNA genome and devoid of an envelope excluding rotaviruses, which are double-stranded RNA, adenoviruses and parvoviruses which are DNA viruses, and the coronaviruses, which contain an envelope. Several virions replicate inside nucleus of the host cell, while the others can replicate in cytoplasm.

Overview of Analytical Methods

Recently, both conventional as well as modern detection methods are available for food and environmental virological analysis. Microbial culture and plaque assay are among the primary diagnostic techniques of microbiology, used as a tool to determine the enormity of viruses by multiplying the agent on a known culture media under controlled laboratory conditions. However, standard culture conditions for many important enteric pathogens are yet not optimized (Mattison and Bidawid 2009). In addition, viral cultures require appropriate eukaryotic host cells for their multiplication events. Alternatively, electron microscopy has been used to detect and visualize the virus particles, but it suffers from the low sensitivity problem and requires high virion titer in sample. Specific diagnosis of rotavirus A and astroviruses are made by the enzyme immunoassay methods (Guix et al. 2005; Wilhelmi de Cal et al. 2007). Many licensed test kits are available in the market which are sensitive, specific and can efficiently detect all the serotypes of rotavirus A (Beards et al. 1984; Smith et al. 1993). Other methods include polyacrylamide gel electrophoresis technique, but it is commonly applied for the diagnosis of viral infection in research laboratories (Beards 1988). Molecular biology tools have the extreme sensitivity and confine the excellent specificity. Polymerase chain reaction (PCR), reverse-transcription PCR (RT-PCR), real-time PCR or real-time RT-PCR, nucleic acid sequence-based amplification (NASBA), real-time NASBA and loop-mediated isothermal amplification (LAMP) or reverse-transcription LAMP can detect and identify all the species and serotypes of the human rotavirus and NoVs (Fischer and Gentsch 2004; Marshall and Bruggink 2006; Gabrieli et al. 2009). In case of the molecular detection methods, nucleic acids (DNA/RNA) need to be extracted from the viral concentrates by either manual (Baert et al. 2008; Kim et al. 2008) or commercial kit-based methods. Serological tests identify antibodies in the serum that are typically produced by programmed response against viral infection (Ryan and Ray 2004; Panda et al. 2007). Unluckily, the PCR-based tools do not persistently amplify nucleic acids if viruses are found in infected food or environmental samples at critically low level. All these analytical tools suffer with the most challenging problem of sample preparation (Mattison and Bidawid 2009).

Biosensor Technology for Virus Detection

A fine quality biosensor device can respond to certain property of analyze (virus nucleoproteins) and transform the response(s) into a detectable signal, often an electric signal. Biorecognition elements specifically identify and interact with analyte to bring about certain changes in their physico-chemical properties such as optical, electrical, thermal, and thermodynamic properties. Further, change in physico-chemical property is converted into electrical signal by the transducers. This signal may provide direct information about the measurable quality factor(s) or may give a known relation to it as illustrated in Fig. 1. A diverse range of biorecognition elements such as nucleic acids, proteins, enzymes, antibodies and several other biopolymers may be recruited for fabricating biosensor assembly. The biological and physico-chemical components of biosensors are kept in close proximity by immobilization processes in order to ensure an unlimited access of sensing elements to the analyze. Biosensors are generally categorized into several groups, according to the signal transduction and to the biorecognition principles. On the basis of the transducing element, biosensors can be regarded as electrochemical, piezoelectric, potentiometric, amperometric, calorimetric, optical, acoustic, and immunosensors.

**Fig. 1** The main parts of a typical biosensor
Among them, the electrochemical, amperometric and the potentiometric biosensors are the extremely exploited ones; optical transducers are the next most frequently used techniques. Indeed, the majority of catalytic biosensors are based on electrochemical principles, yet affinity biosensors have been highly amenable to optical detection protocols (Turner 2000). Mostly, optical transducers make use of the physico-chemical properties such as simple light absorption, refractive index, fluorescence/phosphorescence, bio/chemiluminescence, reflectance, and Raman scattering to bring about the analyte detection (Collings and Caruso 1997). Another successful innovative biosensor with combined microfluidics and biosensing capabilities, furnish real time and automated affinity bioanalysis (e.g. for antigen–antibody assays) through surface plasmon resonance (SPR)-based original optical transduction mechanism. It is a highly sophisticated and expensive device based on the laser excitation, self assembled monolayers and the microfluidics techniques.

Sample Preparation for Biosensor Analysis

Sample preparation is a critical step in biosensor-based microanalytical protocols. Various biosensors detect analytes (virion nucleoproteins) in a small but relatively clean sample. Direct amplification of viral genes through PCR and NASBA are sensitive to inhibitors present in food samples such as vitamins, proteins, other small molecules and hence entail extensive sample preparation and cleanup protocols. Large volume of a food or environmental sample must be processed prior to analysis in order to detect low levels of pathogenic viruses. Biosensors require only a microliter or even much smaller volume of prepared sample for detection of the virion nucleoproteins. Diverse forms of food matrices make it difficult to develop a universal sample preparation method. In case of solid foods, buffer diluted solutions are homogenized to obtain a homogeneous mixture for further processing and detection of virion elements. Laser-induced cell lysis system can efficiently rupture viral cells and release nucleic acids in the sample, followed by amplification of viral genes through PCR and NASBA (Wu et al. 2001). With the advancement in technologies, biosensor gets miniaturized and necessitates inclusion of a sample concentration step. Although, a pre-enrichment step is time consuming and makes quantification of the actual microbial load impossible, it has been a reliable method to increase count of microorganisms in a sample for easier detection (Jaykus 2003). Immunoﬂuorescent separation (IMS) method can efficiently separate and concentrate viruses from the food matrix. IMS employs antibodies immobilized on micron-scale paramagnetic beads to capture and isolate analytes from an aqueous food and environment systems. Antibodies are highly capable of binding the analyte even at low concentrations. Involvement of IMS with standard culture methods for food and environmental virology is found to be a sensitive approach that reduces the detection time (Jackson et al. 1993). Automated IMS systems have great promise for isolating pathogens from various food systems including fresh salad (Prentice et al. 2006), ground beef (Wu et al. 2004), and potato products (Warren et al. 2006). Other approaches such as buoyant density gradient centrifugation can also be successfully used for separation of viruses from food matrices before PCR amplification of nucleic acids (Fukushima et al. 2007).

Biosensor Recognition Elements in Virion

Being subcellular agents, viruses are unable to multiply outside the host cell, and they enjoy common basic structure including one type of the nucleic acid (DNA or RNA) and a protective protein coat often referred to as capsid. The nucleic acid carries the genetic information needed to program the synthetic machinery of the host cell for viral replication whereas the capsid performs two functions: (a) it protects nucleic acid from environmental nucleases action, and (b) facilitates attachment of the virion to host cell, which is, otherwise, negatively charged and repels naked nucleic acid. Slightly more complex arrangements are present in those viruses which infect animal hosts. They additionally acquire biopolymer envelope (glycoproteins, lipids) and enclose essential enzymes for their replication as depicted in Fig. 2. Human immunodeﬁciency virus (HIV) has an additional lipid envelop, glycoprotein

![Fig. 2 A sketch of general anatomy of human immunodeﬁciency virus (HIV)](image-url)
surroundings and reverse transcriptase enzyme to meet replication requirement. Since molecular recognition trait is central in the biosensing systems, all the structural components can be targeted to device a biosensor for detection of the specific virion particles present in food and environment samples. Molecular nanotechnology-based new nanostructures/nanomaterials such as aptamers are capable for developing highly specific biosensor for target elements detection. Various nanomaterials are highly specific for the viral structural components including nucleic acids and proteins. Some aptasensors can detect the specific nucleotide sequences of viral nucleic acid, while the others can identify protective coat biopolymers.

Virology often involves the sensitive and selective detection of specific nucleoprotein structures associated with a particular virion. It is based on the mutual dependence of the organized structure of immobilized nucleic acid probes with the viral DNA/RNA that results in the alteration of the energetics and kinetics during nucleic acid hybridization. Such highly specific, base-pairing interactions can trigger thermodynamic changes that are detectable to sensors. The behavior of the biosensor device significantly depends on the synthetic quality, density and the uniformity of distribution of probe molecules and the free energy of the substrate surface. Several detection methods have been highly sensitive for the nucleic acid identification and analysis. An osmium-based polymeric electrochemical indicator (Liu and Anzai 2004) and electrochemical method based on electrocatalytic oxidation of nucleobase residues (Gore and al. 2003) constitute eminent biosensing strategies. DNA-based biosensors have great applications in food and environmental analysis including determination of the pathogenic bacteria (Liao et al. 2006), and virus DNA sequence such as that of SARS virus (Abad-Valle et al. 2005). Recent diagnostic approaches of the biosensors and the nano-biotechnological assemblies used in identification of pathogenic viruses are briefly summarized in Table 2. Electrochemical biosensing of viral DNA is a rapid and the inexpensive diagnostic approach. Such electrochemical biosensor has been developed for detection of human papilloma virus (HPV). The electroactivity of nucleic acids for electrochemical sensing can be determined by use of methylene blue (MB) dye that intercalates in the DNA double helix during the course of hybridization. Upon incorporation in hybridized DNA, MB generates voltage deflection signal that is voltametrically detectable through biosensor (Sabzi et al. 2008). Another tool for detection of hybridized dsDNA immobilized at the fibre surface is use of the fluorescent DNA stain ethidium bromide (EB). In such cases, the dsDNA hybrid comprised of ssDNA of virion or its RT–PCR amplified cDNA and complementary probe DNA present in bound state to the fibre surface. Detection of the dsDNA hybrid can be achieved by treating the complex to an EB staining solution followed by washings with the hybridization buffer solution. Strong association existing between dsDNA hybrid and the ethidium cation (3,8-diamino-6-phenyl-5-ethyl-phenanthridium) can be monitored through total internal reflection pattern along an optical fibre to quantify the presence of dsDNA hybrid at the surface of the fibre. The fluorescence intensity virtually related to the quantity of either ssDNA or RT-PCR amplified cDNA present in the solution. This approach can further be improved and applied for rapid identification and quantization of the microbial contaminants such as pathogenic bacteria and viruses in environment, food and feed commodities (Piunno et al. 1995).

Gold nanoparticles equipped with fluorescence resonance energy transfer (FRET) principle can detect porcine reproductive and respiratory syndrome virus (Grant et al. 2006). A photoactivatable, electrogenerated poly(pyrrrole-benzophenone) film deposited on indium tin oxide (ITO) plates can modify the surface optical properties of conductive fibres. Such photo immobilization methodology has been applied to detect antibodies directed against antigens of two Ebola virus strains Zaire and Sudan (Petrosova et al. 2007). Similarly, poly(pyrrrole-benzophenone) film-based optic biosensors are employed for the diagnosis of hepatitis C virus antigens (Konry et al. 2005). Quartz crystal microbalance (QCM)-based piezoelectric sensors can detect the hybridized viral DNA and also the capsid protein–ligand interactions. (Cooper et al. 2001). The bioanalytical application of QCM has been shown for influenza A virus, M13-phage virus, Cymbidium mosaic potexvirus, and dengue virus (Sato et al. 1996; Uttenthaler et al. 2001; Eun et al. 2002; Su et al. 2003). Charych et al. (1993) have reported a colorimetric sensor composed of a polydiacetylene bilayer assembled on glass slides for detection of influenza virus. A fluorescence-based biosensor has capability of simultaneous analysis of the multiple samples for multiple agents/analytes. The patterned groups of antibodies immobilized on the surface of a planar waveguide can be employed to capture diverse analytes present in the samples. Such sensors referred to as planar array immunosensor that has been integrated with a charge-coupled device (CCD) detector and a diode laser as light source. Certain modified planar immunosensors can be developed and applied to the simultaneous determination of multiple virion-particle elements (Rowe-Taitt et al. 2000).

Recently, the aptamer-based biosensors are becoming increasingly popular. Unlike traditional nucleic acids, aptamers are synthetic, single-stranded nucleic acids that
| Virus                                      | Transducer   | Recognition element                  | Nanomaterial/nanotechnology                  | References               |
|-------------------------------------------|--------------|--------------------------------------|---------------------------------------------|--------------------------|
| African swine fever virus (ASF)           | Piezoelectric | Virus protein                         | Quartz crystal microbalance (QCM)           | Uttenthaler et al. (1998) |
| Avian influenza virus                     | Interferometric | Viral protein                        | 3-Mercaptopropyltrimethoxysilane           | Xu et al. (2007)         |
| Avian leucosis virus (ALV)                | Optical      | Viral antigen                         | Germanium-doped silicon dioxide             | Huang et al. (2006)      |
| Cymbidium mosaic potexvirus (CymMV) and Odontoglossum ringspot tobamovirus (ORSV) | Piezoelectric | Viral antigen                         | Quartz crystal microbalance (QCM)           | Eun et al. (2002)        |
| Cytomegalovirus                           | Piezoelectric | Viral antigen                         | Thiol/poly-L-lysine                        | Susmel et al. (2000)     |
| Dengue virus                               | Voltammetric | Oligonucleotide sequence              | Chitosan derivatives                        | Teles et al. (2007)      |
| Dengue virus                               | --           | Viral RNA                             | --                                          | Baeumner et al. (2002)   |
| Dengue virus                               | Amperometric | Viral RNA                             | --                                          | Kwakye et al. (2006)     |
| Dengue virus                               | Piezoelectric | Viral envelope and non-structural protein | Quartz crystal microbalance (QCM)           | Su et al. (2003)         |
| Dengue virus                               | Fibre-optic  | Viral RNA                             | Acetylthioacetate-tagged liposomes          | Zaytseva et al. (2004)   |
| Ebola virus                                | Fibre-optic  | Viral antigen                         | Poly(pyrrrolebenzophenone)                 | Petrosova et al. (2007)  |
| Ebola virus (EBOV)                         | Optical      | Virus envelope                        | Surface plasmon resonance and a quartz crystal microbalance | Yu et al. (2006)         |
| Hantavirus                                 | Amperometric | Viral antigen                         | --                                          | Krishnan et al. (1996)   |
| Hepatitis B virus (HBV)                    | Amperometric | DNA sequence                          | Co(1,10-phenanthroline)$^{3+}$             | Erdem et al. (1999)      |
| Hepatitis B virus (HBV)                    | Optical      | DNA sequence                          | Magnetic beads                             | Chen et al. (1998)       |
| Hepatitis B virus (HBV)                    | Piezoelectric | Viral antigen                         | Quartz crystal microbalance (QCM)           | Zhou et al. (2002)       |
| Hepatitis C virus (HCV)                    | Electrochemical | Virus replicase and helicase         | Aptazyme                                   | Cho et al. (2005)        |
| Hepatitis C virus (HCV)                    | Optical      | Viral antigen                         | Poly(pyrrrole-benzophenone)                | Konry et al. (2005)      |
| Hepatitis E virus (HEV)                    | Affinity     | Capsid protein                        | Carboxylated dextran                       | Schofield et al. (2000)  |
| Herpes simplex virus (HSV)                 | Electrochemical | Viral antigen                        | Screen-printed carbon electrode            | Kelso et al. (2000)      |
| Herpes simplex virus (HSV) I and 2, Varicella-Zoster virus (VSV), Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) | Piezoelectric | Viral antigen                        | --                                          | Koenig and Graetzel (1994) |
| Herpes simplex virus type 1 (HSV 1)        | Acoustic     | Viral antigen                         | Quartz crystal microbalance (QCM)           | Cooper et al. (2001)     |
| Human immunodeficiency virus               | Amperometric | DNA sequence                          | Co(1,10-phenanthroline)$^{3+}$             | Wang et al. (1996)       |
| Human immunodeficiency virus (HIV-1)       | Piezoelectric | HIV-1 transcription trans-activator (Tat) protein | Aptamers                                   | Minunni et al. (2004)   |
| Influenza A virus                          | Piezoelectric | Capsid protein                        | Glucosylceramide and Sphingomyelin         | Sato et al. (1996)       |
| Influenza virus                            | Optical      | Viral envelope                        | Carboxylated dextran polymer               | Schofield and Dimmock (1996) |
| Influenza virus                            | Colorimetric | Viral envelope                        | Polydiacetylene, Sialic acid               | Charych et al. (1993)    |
| Influenza virus                            | Colorimetric | Viral envelope                        | Polydiacetylene liposomes                  | Anke et al. (1995)       |
| Influenza virus                            | Colorimetric | Viral antigen                         | Polydiacetylene liposomes                  | Reichert et al. (1995)   |
| Porcine reproductive and respiratory syndrome virus | Fibre-optic   | Viral antigen                         | Gold nanoparticles and FRET                | Grant et al. (2006)      |
| Rift valley fever virus (RVF)              | Fibre-optic  | Viral antigen                         | --                                          | Sobarzo et al. (2007)    |
| SARS virus                                 | Amperometric | DNA sequence                          | --                                          | Abad-Valle et al. (2005) |
| SARS virus                                 | Imaging      | Viral envelope                        | 3-Aminopropyltriethoxy-silane              | Qi et al. (2006)         |
exhibit specific ligand-binding affinity against a wide range of molecules such as amino acids, drugs, proteins and other small biomolecules. Structural studies have revealed that aptamers can frequently undergo significant conformational modifications upon binding to their cognate ligands (Hermann and Patel 2000; Patel and Suri 2000). These structural changes can be identified by several strategies involving the fluorophore-based optical reporters, nanoparticle reporters, electrochemical detection, and the mass-sensitive detection approach (Cho et al. 2009). Aptamers can be raised against any virion structural component through the systematic evolution of ligands by exponential enrichment (SELEX) process from random RNA or DNA libraries (Tuerk and Gold 1990; Ellington and Szostak 1990). Viral polymerases were the earliest opted targets for aptamer technology. Lateron, it has been showed that the aptamers can target a wide range of molecules which appear during viral life cycle in extracellular as well as intracellular phases as depicted in Fig. 3. Tuerk et al. (1992) have isolated RNA aptamer against the reverse transcriptase enzyme of human immunodeficiency virus 1 (HIV-1) and applied for their identification. Similarly, a potent DNA aptamer has also been isolated against hepatitis C virus (HCV) polymerase (Biroccio et al. 2002; Bellecave et al. 2003). Further, chip-based RNA aptamers can be used to recognize the structural proteins of HCV and to bind its core antigens E1/E2 (Lee et al. 2007). Since aptamers have high thermal stability, long shelf-life, and the ease of chemical synthesis and modification, in addition to their affinity and specificity which rival those of antibodies; therefore, they have been proved to be the useful viral nucleoprotein detection and confirmation tools (Jayasena 1999).

New Trends in Biosensor Development

Synthesis of novel smart materials and the nanostructures open wide horizon for biosensor technology. Advancement in micro- and nano-assemblies leads to improvement in the capabilities and affinities of biosensors for viral element detection. The sialic acid ligand serves as a molecular recognition element. This specific nature of sialoside film facilitates its interaction with influenza virus which is detectable through a colorimetric competitive inhibition assay readily visible to unaided eyes (Charych et al. 1993). Polydiacetylene liposomes embedded sialic acid serve as the high affinity recognition elements against virion nucleoproteins in comparison to the basic liposomes (Reichert et al. 1995). A color-shifting glycopolythiophene sensor that contains sialic acid as the receptor and a fluorescent polymer as the transducer has been reported for virus detection at the zeptomole level (Leclerc 1999; Baek et al. 2000). Analyte detection through the magnetic-particle-label is very fast and the robust process. Magnetic markers specifically label the analyte and allow it to settle at the bottom of a vial. These sediments of analyte with magnetic markers can be identified by biosensors and their abundance can be translated into a quantifiable electronic signal (Larsson et al. 1999). A new detection method for molecular recognition of viral element’s reactions is based on the magnetic markers and the magnetoresistive sensors. The magnetic markers can specifically bind to a biomolecule whose magnetic microsphere can be identified as a change of electrical resistance through embedded magnetoresistive sensors (Schotter 2004). Another emerging group of biosensors are the cantilever biosensors, which examine the extent of bending of the silicon cantilevers as

| Virus                         | Transducer | Recognition element | Nanomaterial/nanotechnology                  | References         |
|-------------------------------|------------|---------------------|---------------------------------------------|--------------------|
| SARS-associated coronavirus (SARS-CoV) | Piezoelectric | Viral antigen | –                                    | Zuo et al. (2004)  |
| Vaccinia virus                 | Fibre-optic | Viral antigen       | Streptavidin-coated polystyrene           | Donaldson et al. (2004) |
| Vaccinia virus                 | Fibre-optic | Viral DNA           | DNA array                                | Song et al. (2006)  |
| West Nile virus (WNV)          | Amperometric | Viral antigen      | –                                        | Ionescu et al. (2007) |
a result of adsorption of target molecules onto the cantilever surface, previously immobilized with the receptor molecules. Moreover, a screen-printing technology has enabled the fabrication of modern biosensors which can be integrated in the tiny portable systems. The biosensors based on screen-printed electrodes underwent chronological evolution and has a large, practical impact on environmental and food-analysis applications (Tudorache and Bala 2007).

Recently, a microfluidic biosensor module has been developed for the identification of viral nucleoprotein as a model analyte. Microfluidic devices integrate the sample handling, reagent mixing, separation, and the detection processes together. A well-known technology of lateral flow RNA assay is adapted to the microfluidic system. The system is devised in modular form in which all the operations required for analysis such as cell lysis, RNA purification, nucleic acid sequence-based amplification (NASBA) and RNA detection, are performed in separate modules. The modules combined together will compose the microsystem. The microfluidic biosensor consists of a network of microchannels fabricated in polydimethylsiloxane. Computer-aided design software has been used for modelling of microfluidic biosensors and they are nanofabricated in the silicon wafers. Such biosensors often identify the specific messenger or genomic RNA of viruses and devoid of a separate sample preparation step (Zaytseva et al. 2005).

Although, aptasensors came into existence during the past decade only, they have flourished tremendously in both basic virological research and the biomedical diagnostics. Diverse range of transducers (e.g., acoustic, electrochemical, optical and mass-sensitive) can be employed in aptasensors. Particularly, label-free sensing formats (e.g., SPR, QCM, surface acoustic wave and micromechanical cantilevers) offer the promise of reagentless, single-step detection of virion nucleoproteins (Mok and Li 2008; Song et al. 2008). In last two decades, several researchers have reported the immobilization of aptamers and aptasensors onto nanomaterials such as cellulose (Su et al. 2007), microgels (Su et al. 2008) and xerogels (Holthoff and Bright 2007). This paves the way for miniaturization of biosensors and the production of portable sensors from the food virology and the disease diagnostics viewpoint.

**Conclusion and Future Prospects**

Conventional technologies need about 7 days of microbial detection time, since water treatment plants and food industries have to send their sample to a testing lab and pay about $400 per analysis. Alternatively, an estimated cost for a biosensor-based method can be $25 per analysis for the water sample. Furthermore, a biosensor for detection of dengue virus has been designed that can distinguish among all the four serotypes. New nanotechnological advancements including integrated systems and the use of molecular beacons can move the analytical microsystems into the micro- or nano-biosensor area by miniaturization techniques that decrease the detection time and also make them completely portable. Although their commercialization process is slow, still biosensors are making a great impact on everyday life and, indeed, will find their application in the developing world.

With a $500 million-per-year market, the biosensor based on DNA probe technology is the fastest growing, in vitro diagnostic market and is estimated to grow at annual rate of 25% during the upcoming few years. Worldwide, about 89 companies take over the primary and secondary research on biosensors including many key and niche players. Success or failure of detection technologies is principally determined by improvement in the affinity, specificity, and mass production of the molecular recognition components. Since, scientific attention is currently focused to nanobiotechnology-based sensors, because it plays a pivotal role in the generation of smart nanomolecules with predefined properties, such as selectivity, affinity and stability. The advanced biorecognition molecules will allow the analysis of complex real samples and in situ measurements resolving the faults of nonspecific background noises. Conclusively, the demand for rapid and real-time microanalytical biosensors will only increase as the world has now become more concerned about the fact that food and environmental contamination may afflict the public health and the ecosystem.

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