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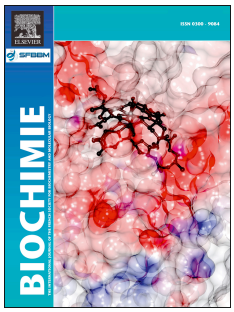

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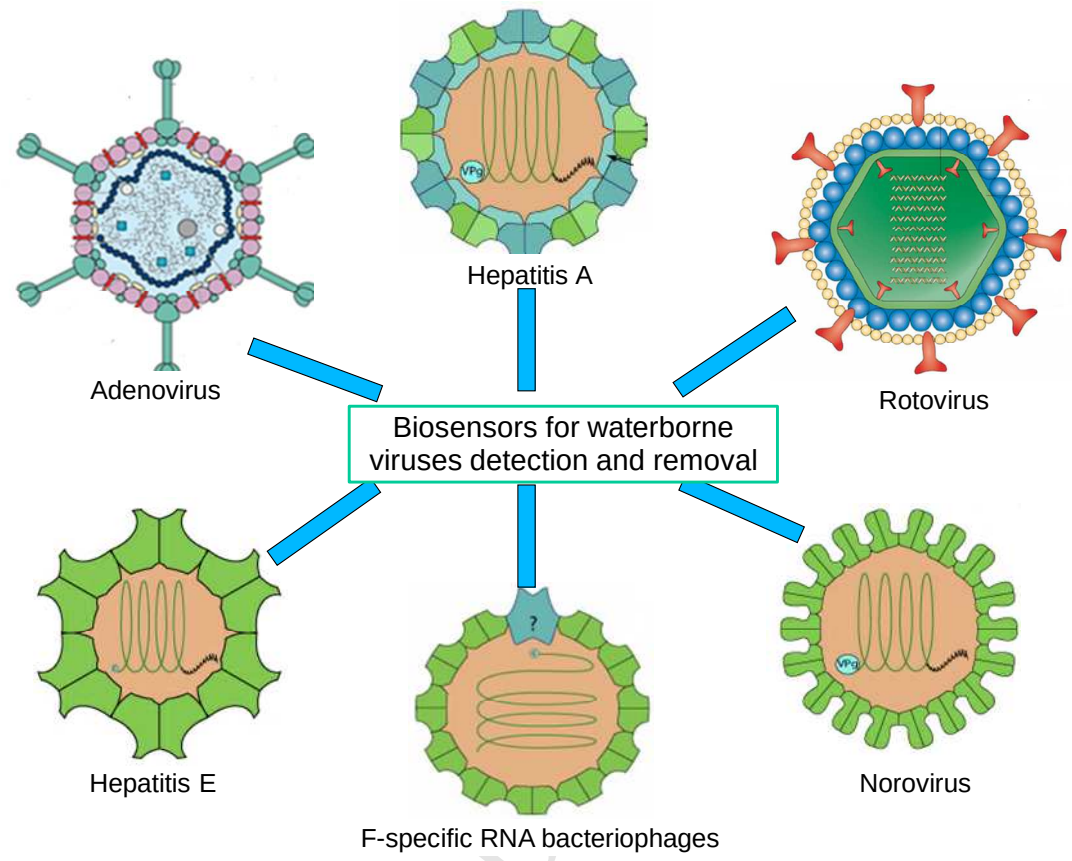
Graphical Abstract



Biosensors for Waterborne Viruses: Detection and Removal

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Abstract

Detection of waterborne viruses is important to eliminate and control their harmful effect as pathogens. Hence, the use of rapid and sensitive detection technologies is critically important as they can aid in investigating outbreaks and help in developing prevention strategies. To date range of viruses can contaminate drinking water sources, causing illnesses such as diarrhoea, pneumonia and gastroenteritis which can result in death. Due to their small size (nm) their complete removal from water can be difficult with current water treatment processes while being resistant to disinfectants. Available techniques for virus detection include filtration technologies, enzyme-linked immunosorbent assays and polymerase chain reaction. Although each technique has limitations, the use of biosensor technology with smart affinity materials and nanomaterials can show great potential in sensing viruses in water samples. This review reports on the latest technologies used for waterborne virus removal and detection with focus on rapid detection using biosensors.

Keywords: Virus detection; Biosensor; Waterborne diseases; Affinity-based capture.

1. Introduction

Approximately 884 million people lack access to sanitary, safe drinking water which accounts for roughly 13% of the global population [1]. This results in millions of people dying every year due to the contraction of waterborne diseases. Therefore, increasing access to safe, sanitary

drinking water can significantly reduce deaths and increase global public health. Efforts must be taken to ensure that drinking water is not contaminated with harmful biological agents. Common disinfection techniques include the use of chemical oxidants (chlorination), UV irradiation and thermal treatment. Generally virus inactivation methods have not been successfully developed; currently this is due to the numerous waterborne virus strains which are not culturable in vitro and thus cannot be investigated and characterized. The susceptibility of many waterborne viruses to inactivation is relatively unknown because of the inability to culture these viruses [2]. Viral diseases can be preventable but unfortunately account for hundreds of thousands of deaths each year, many of them being young children under five years of age [3-4].

Viruses have diverse mechanisms of action and consist of a virion (10-100 nm) containing DNA or RNA genome encapsulated in a protein coat [5]. The function of viral nucleic acid is to carry the genetic information required to program the synthetic machinery of the host cell for viral replication whereas the capsid protects the nucleic acid from nucleases and aids in the attachment of the virus to the host cell. Viruses require a host organism to reproduce and many are pathogenic. The enterically transmitted viruses hepatitis A, hepatitis E, norovirus and rotovirus are the major causes of waterborne diseases in humans. These viruses are infectious in low doses (generally only a few virion particles are required for infection), stable in the environment for extended periods of time and difficult to study. Viruses detection in water require specialized analytical methods and experienced personal. This combined with the detection limits of the analytical techniques used are insufficient for researchers to conclude that a sample is completely free of viruses. No known diagnostic test offers complete assurance that a sample is 100% free of viruses [6]. Also viruses are highly resistant to acidic conditions, high temperatures, disinfectants and pressure. They are intracellular parasites incapable of reproducing outside of the host's cells. Once they infect the host cells they direct the production of vast quantities of virus progeny which are excreted by the infected organism and which pose a threat to other healthy organisms [7]. The human enteric viruses enter the general water supply through contaminated sewage waste [8]. The viruses are so prevalent in sewage because infected humans excrete the viruses in enormous quantities. Persons infected with these viruses can excrete 10^5 to 10^{11} virus particles per gram of faeces. Viral infections are difficult to effectively diagnose and often diagnosis is only determined when the virus has spread to other individuals [9-11].

The currently available techniques for virus detection and/or removal are enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), different types of filtration techniques and biosensor technology. Although each technique has its own limitations, rapid detection methods based on biosensors provide the best opportunity since they are easy to use, cost-effective, sensitive and generally real-time detection methods. The latest developments in nanotechnology and the design and production of sensor surfaces based on synthetic materials that can be used as sensing receptors has shown great potential in achieving low detection for viruses sensing. In this paper, we review the trends in waterborne viruses detection.

2. Waterborne viruses

There are different types of waterborne viruses including hepatitis A, hepatitis E, rotavirus, adenovirus, norovirus and F-specific RNA bacteriophages (**Table 1)**. Hepatitis A is transmitted to humans via the faecal-oral route or direct contact with the infected person. It mainly affects the developing world, particularly areas with poor sanitation. Vaccination is usually effective with this type of virus. Mortality rates for infection from the disease is very low, approximately 0.5% [12]. The virus enters the body via the oral route and then multiples in the intestines before spreading to infect the liver [13]. The virus is resistant to detergents, organic solvents, acidic conditions, desiccation and temperatures up to 60°C, while susceptible to UV radiation and chlorine treatment. Infection can be reduced by vaccination, excellent hygiene and sanitation. Hepatitis E causes more severe disease than hepatitis A and is genetically unrelated to it. There are four global genotypes with the most virulent, genotypes 1 and 2, present in the developing world [3]. Outbreaks or epidemics associated with hepatitis E are attributed to the faecal contamination of drinking water [7]. The virus causes inflammation of the liver which is not fatal but results in incapacitating pain, vomiting and fever [12].

Rotavirus is responsible for a large number of diarrheal deaths especially in children. Infections with rotavirus are more frequent in the winter months and cause 5% of all child deaths annually, particularly in the developing world [3]. There are seven different groups of rotavirus

which makes vaccination against it difficult [3, 7] and the sanitation methods are generally ineffective [12]. Adenoviruses are responsible for respiratory, ocular, and enteric infections as well as pneumonia [13]. The viruses are difficult to diagnose as they produce few symptoms to indicate infection. Currently, human adenoviruses are classified into 54 serotypes, and placed into seven subcategories, labelled A-G [14]. Transmission mainly occurs via respiratory droplets but the virus is also transmitted via the oral-faecal route. Noroviruses are non-enveloped common agents of gastroenteritis which leads to significant fluid loss and can be fatal if adequate treatment is not provided [7, 13]. Transmission occurs via fecally contaminated food and water, person to person contact, contaminated surfaces and via aerosolization making the virus highly dangerous. The virus is resistant in the environment and is especially resilient in bodies of water [12, 15]. As the last group of waterborne viruses, bacteriophages (F-specific RNA bacteriophages) are naked RNA viruses with encapsulated protein capsids with a size ranging from 21-30 nm. The genetic codes of these phages vary in length but not significantly. Bacteriophages are viruses that infect and replicate within bacteria. They are popularly used in virus research since they are easily manipulated and are not pathogenic to humans. The phages are used as surrogates in the evaluation of the efficiency and effectiveness of water treatment systems. This is due to their small size and their similar structure to human pathogenic viruses. Bacteriophage MS2 is the most popular surrogate of these phages [16-18]. **Figure 1** shows the common schematic structures of waterborne viruses [12].

3. Separation, filtration and affinity recognition of viruses

To this day, eliminating viruses from water is a very challenging task using present-day water-treatment methods. This is due mainly to the unique properties of viruses, such as their small size making them difficult to filter (~20-90 nm), as well as their increased resistance too many disinfection techniques that are routinely used to kill other pathogens. Because of these challenges, there is greater focus on developing more sophisticated methods for removing viruses from water [19-22]. Virus filtration is generally reliable for virus removal, but there are many issues to consider when designing a novel retention filter. The filter system must take into consideration the viruses size are at nm scale. The filter

process and the filter's composition should be tailored to ensure efficient viral removal [20]. With the increasing cost of energy there is a need for filtration systems to be less energy intensive and more efficient with increased capture of contaminants. The maintenance of these filters must also be economical, practical and relatively non-intensive. Modern filtration methods are focusing on viral inactivation during filtration because of the advantage of killing the viral agents. Inactivating viruses will ensure that there is no chance of the water being re-infected by the virus during filter maintenance or viral particles escaping through the filter to contaminate already processed water [23]. Viral inactivation is highly variable between virus types, types of treatment and the environment which the virus exists in. This is because of the different physical and chemical properties of viruses' protein coats [20, 24].

3.1. Molecular Weight Filtration

The global installed capacity of low - pressure membrane increased 10 folds during 1996 to 2006 to over 3 billion gallons per day, with 60% and 22 % of this applied in drinking water and waste water respectively [19]. Modern filters have demonstrated high virus retention efficiencies, however, their success is heavily influenced by the biological variability of viruses. Membrane filtration is a popular technique to filter viruses as membranes are able to process large volumes of water, and are able to remove viruses from water out of too many organic contaminants present [25]. Their efficiency is reduced when high levels of organic matter are present in the water. This is due to the preferential adsorption of the organic material that enables viruses to pass through untreated into the filtrate. Where organic contaminants are a problem, these can be removed by pre-filtration usually combined with trivalent aluminium salts in order to assist in the adsorption of viruses to electronegative filters – although this acidifies the treated water and adds an extra step to the process [26]. Processing volumes of water in excess of 1500 L causes the clogging of filters with particulate matter. While electronegative filters are effective at removing viruses from water they require acidification of the processed water or the addition of polyvalent salts which is a significant disadvantage when processing large volumes of water. Electropositive filters overcome the restrictions which are required with the use of electronegative filters. Electropositive filters have a coating of

Al^{+3} ions which allows adsorption of viruses over a wider range of pH. However, a pre-concentration step may still be required when processing large volumes of water as organic matter may inhibit the filter's processing ability. Glass wool is also a material used to remove viruses from water due to its positive charge and its hydrophobicity. It is inexpensive and can process large volumes of water [26].

Ultrafiltration is an alternative virus filtration technique that uses size-exclusion rather than electrostatic attraction to partially charged filters [27]. A significant advantage of ultrafiltration is that no pre-conditioning is required; however, it is an expensive technique and requires trained operators. During this procedure, viruses and other contaminants may adsorb onto the membrane via van der Waals forces or hydrophobic interaction. To combat this, blocking solutions (sodium polyphosphates) are applied to the membrane. Several types of ultrafiltration methodologies are currently in place which are differentiated by the flow rates utilised to separate particles by their molecular weight. The flow can be either dead-end flow or various types of cross-flow (including tangential flow and vortex flow). Filter geometries also differ such as spiral wound, tubular, and hollow fibre membranes, and the infrequently used plate and frame membranes. Spiral wound membranes have layers of filter material wound around a central permeate collection tube. These membranes are inexpensive and therefore very widely used in the water industry. Tubular membranes have wide central channels where the water passes through. Hollow fiber membranes allow the processed water to pass through the membrane fibres where the filtrate is collected. The water can flow through the membrane from inside to outside or from outside to inside [26]. The pore sizes of ultrafiltration membranes vary between 1 and 100 nm, similar to the size range of enteric viruses, so they have potential for virus removal [28], unlike the common bacteria-removal technique microfiltration, which uses symmetric or asymmetric membranes with pore sizes between 0.1-10 µm – too large to retain smaller viruses.

Reverse osmosis is a common process used in ultrafiltration, using a flat asymmetric thin-film cellulose acetate or polyamide membrane with a designated molecular weight cut-off 9 ~ 100 Da). Unlike ultrafiltration membranes, reverse osmosis membranes are nonporous, and also typically reject the vast majority dissolved salts in water. However, these membranes are prone to random incidences of viral penetration leading to sporadic differences in virus removal rates – not ideal when trying to hit the WHO's target of 99.99% consistency. A superior alternative is the use of hollow-fibre ultrafilters composed of asymmetric polysulphone fibres, as they reduce the loss of viruses due to the dense inner skin lining in

direct contact with sample feeds. The filters are also less susceptible to the effects of pH and temperature fluctuations and bacterial degradation. Hollow fibre filtration membranes are composed of poly-sulfone fibres or polyacrynitrile fibres and as a result are generally efficient with filtering viruses [26]. Tangential flow filtration is a cross-flow process in which the processed water is fed tangentially along the surface of the membranes. The rapid flow of fluid across the membrane creates a pressure differential which causes any particles smaller than the membrane pore size to pass through the membrane and into the filtrate container. Viruses are unable to pass through the filter. The rapid flow of liquid prevents the accumulation of particulates and the fouling of the membranes and hence is effective in processing low quality water. Tangential flow apparatus is expensive and requires large stainless steel equipment which is immobile and impractical to move from the laboratory. Dead-end filtration allows for the filtration of aqueous media in the field. The water to be processed only flows through the filter once. The apparatus is easy to set up and easy to construct on site [26]. While microfiltration and ultrafiltration have been effectively used as robust method of virus reduction, they are not 100 %. Current ultrafiltration membranes still allow some viral particles to permeate the membrane, usually through abnormal sized membrane pores. Yang et al. reported the development of nano-porous films which have been used successfully to filter the common cold virus (human rhinovirus type 14). The films have uniform pore sizes because of their fabrication is by self-assembly [29]. **Table 2** highlights the most currently used commercial filters.

3.2. Adsorbent Filtration Materials

Viruses when present in aqueous mediums, they become charged biological particles due to their isoelectric points ranging between pH 3-7. Since natural aqueous environments tend to have a pH value of between 4-9, most viruses in water are negatively charged. Adsorbent filtration materials exploit this property of virus charge; for instance, to filter a negatively-charged virus, a positively-charged surface would be used to adhere the target particles [31]. If the electrostatic forces present on the surface are strong, it is even possible to bind and inactivate the virus simultaneously. The adhesion kinetics of viruses are influenced by pH, aqueous ionic strength, and the presence or absence of interfering

contaminants. These contaminants may either adsorb to the viruses or compete with them for adsorption sites on the filtration surface. A wide variety of materials have been studied as adsorption surfaces such as hematite, ceramic modified materials, clays, activated carbon, and metal oxides/hydroxides [32-35]. All of these materials have been successfully incorporation in filters to remove viruses from water. A key point to consider when designing novel filtration methods is the design of filter coating methods in order to lower the required energy for filtration, as well as regenerate the adsorption surfaces [36]. Hence, filters have also been successfully modified with materials such as magnetite, silver, magnesium oxide and hydrophilic polymers [35-38]. Ceramic water filters which are based on size exclusion have becoming a point- of- use method of contaminant filtration for household use. Even though many of these filters are treated with silver to aid in the inactivation of bacteria [39], they have been reported to shown low viruses removal rate when tested with bacteriophage MS2, since the results were below the WHOs removal targets [39]. However, by implementing magnesium oxide as a positive adsorption surface into ceramic depth filters an improvement in virus removal was observed [35]. Using a filter with a 20% MgO was reported to have greater than log 4 virus retention (>99.99%) after 10 L of water had passed through the filter, but only log 2.5 virus retention after 200 L of the test water had passed through. Therefore, these filters would need to be frequently replaced if used to disinfect drinking water supplies.

Carbon nanotubes are very unique as filters due to their physicochemical properties which include high surface area, electrical conductivity, optical activity, thermal stability/ conductivity and extreme strength. They are also anticorrosive and can be shaped into thin films with micrometre thickness and used adsorption/degradation in water treatment [34]. The removal of viruses by carbon nanotube layers is largely aided by the high surface area and fine pore size of these filters. Two types of carbon nanotubes are currently in use; single-walled nanotubes (SWNTs) [40] and multiple-walled nanotubes (MWNTs) [41]. Research has shown that virus removal by MWNTs is 1.5-3 log higher than SWNTs [42]. Virus removal by adsorption has been shown to be greater when the filtration speed is reduced and also when the ionic strength of the salt in solution increased and the pH is decreased [42-43]. Despite the success of MWNTs at removing viruses, they are sterically hindered by organic contaminants. However, this can be countered by passing a direct current of 2-3 volts through it, taking advantage of the fact that carbon can conduct electricity very efficiently, and improving virus retention rates even when dissolved organic matter is present [34].

Increasing the filter thickness also improves the retention of virus particles in the abundance of organic contaminants. The conductivity of carbon nanotubes also allows for the electrochemical inactivation of viruses, providing them with native anti-microbial properties [44]. Toxicity of carbon nanotubes has to be considered and risks assessed before any widespread application of the technology.

Zerovalent iron has been used in water treatment systems for many years to remove organic and inorganic contaminants [32-33]. In the last decade, nanoscale zerovalent iron has become the subject of increased attention due to its superior surface area and reactivity to its regular counterpart [45]. Since viruses become negatively charged in environmental water, they are able to adsorb to the positively charged iron oxide layer generated by the corrosion of zerovalent iron in water [32]. Iron oxide has also been shown to be able to inactivate viruses through the generation of hydroxyl free radicals. Iron nanoparticles are relatively inexpensive and widely available from chemical reagents. Iron nanoparticles can be incorporated in the design of a point-of-use device for water treatment and is an attractive option because it does not produce harmful disinfection by-products like conventional methods [33,46]. The ideal viral adsorption matrix would have several key characterixtics: adsorb viruses over a wide pH range and environmental water chemistries, achieve a high adsorption capacity, and prevent the release of infective viral particles either through (a) irreversible adsorption, or (b) permanent inactivate [47].

3.3. Affinity Ligands

Affinity ligands are a class of molecular materials which bind specifically to other molecules to form complexes. The binding occurs *via* intermolecular forces such as ionic bonding, hydrogen bonding and van der Waals interactions. Ligands can be specific to one analyte or to a group of analytes, and it is common for ligands to be engineered specifically for a particular target of interest [48]. There are a number of affinity ligands that can be developed specifically towards viruses and these include antibodies, aptamers, and molecularly imprinting polymers (MIPs).

Antibodies are currently the most common recognition materials (receptors) used for the affinity capture and detection of viruses and are naturally produced by plasma cells as part of the immune response to foreign bodies. Two types of antibodies exist; monoclonal and polyclonal and these are used to target foreign biomolecules, binding them very specifically in micromolar to nanomolar concentrations [49]. The high specificity between an antibody and its analyte is exploited in many affinity systems and biosensors [50-51]. Monoclonal antibodies are used in more specific tests than polyclonal antibodies, although they are more difficult to maintain and can be more expensive [52]. Care must be taken to reduce non-specific interactions during the binding of antibodies to target analytes. Antibody-antigen binding can be detected by labelling methods, such as fluorophore/enzyme conjugation or biotinylation [53], as well as by label-free methods, such as by oblique-incidence reflectivity difference (OI-RD) scanning microscopy or using SPR/QCM sensor systems [54]. Antibodies have been demonstrated to have higher specificity than many synthetic affinity molecules in most cases, but carry the caveat of being highly prone to oxidative or microbial degradation, as well as being unusable in harsh environments such as strong acidic/basic conditions or organic solvents [55]. A major advantage of antibodies as recognition ligands is that they have already been developed into highly effective, reliable, and portable sensing systems, such as in pregnancy testing kits to detect the presence of human chorionic gonadotropin (hCG) [56]. Therefore, this has laid a good foundation for future research into similar applications for antibodies. Specificity is one of the most important characteristics of the antibodies for the integrity of any immunoassay to eliminate the potential of cross-reactivity from related analytes. Moreover, the affinity of the produced antibody against the target analyte is critical since it refers to the strength of the bond between the antibody and its analyte. Current antibody production technologies enable the production of very epitope-specific antibodies, which is essential as this is the key component of immunoassay integrity, as false readings can be observed due to an antibody binding a similar antigen not of interest. Antibodies' properties can vary from batch to batch which can hugely hinder their reproducibility. To combat this, strict regulations have been put into place to bring consistency to the manufacture of antibodies, however this may interfere with optimal production processes [57].

Various methods exist for the immobilisation of antibodies to stationary surfaces, such as by using physical adsorption; though a drawback to this is that the antibody's structure can become distorted, affecting its binding kinetics. To avoid this, an alternative method is to covalently bind

the antibody to the surface, as is practised in SPR [58]. Advances in antibody technology have already seen the development of isolated antigen-binding components of antibodies, known as nanobodies, or the development of peptide as diagnostics and therapeutics [59]. Liu et al. [60] successfully developed single-domain antibodies against bacteriophage MS2 and its coat protein, by selecting from an antibody library from llamas immunised with purified coat protein, and a second library from animals immunised with bacteriophage MS2. A range of monoclonal and polyclonal antibodies have been produced for viruses detection in recent years and applied in biosensor technology [61-63]. Figure 2, A –B, gives examples of biosensors based on antibody detection system (64, 65).

Aptamers are synthetic DNA or RNA molecules specifically designed to bind a target analyte of interest. Hence, the name is derived from the Latin word “aptus” meaning “to fit”. They are of great interest in the world of research as they are so diverse in their applications, are flexible and can be designed to bind to small molecules as well as complex targets, such as peptides, proteins, drugs, metal ions and whole cells [66-67]. Production of aptamers is a rapid, automated process, during which random sequences are generated and screened for binding affinity. They can be synthesised with a high level of reproducibility and purity from commercial sources. Aptamers possess high affinity for their targets because they have the ability to fold around their targets upon binding [68]. They can wrap around small molecules or be integrated into the structure of protein biomolecules.

A common screening process for aptamers is the Systematic Evolution of Ligands by Exponential Enrichment (SELEX). During this process, a large library of candidates containing possible sequences and structures is identified and screened sequentially in rounds by incubating the candidate and target molecules. After each round, non-binding candidates are washed off, with the bound candidates being prepared for the next round. Eventually, after around 6-12 cycles, this leads to the enrichment (amplification by PCR) of a small number of high-affinity and high-specificity aptamer clones [69]. The process also allows for the production of aptamers for toxic/non-immunogenic targets, as well as for specific regions of target molecules rather than the whole unit [49].

Many aptamers have been selected for viruses detection. Beier et al. [69] described the binding of DNA aptamers with the sequence 5'-GTCTG TAGTA GGGAG GATGG TCCGG GGCCC CGAGA CGACG TTATC AGGC-3' to norovirus capsids immobilised on a gold sensor chip. Aptamers have been utilised in micro-cantilevers sensors as biological receptors for viruses. Song et al. [66] has described the use of RNA aptamers to detect hepatitis C virus (HCV) helicase. The label-free detection was capable of detecting the viruses at concentrations as low as 100 $pg.ml^{-1}$ HCV helicase. Aptamers have also been designed as therapeutic targets with some designed to block the infectivity of Rhinovirus [48]. They possess such high affinity that their dissociation constants (K_d) for their targets range from nano-molar to pico-molar. They can also be highly selective that they can distinguish enantiomers or molecules which are very similar to each other [49]. Aptamers can be easily large-scaled due to their reproducibility, and recent technological advancement has led to aptamers becoming incorporated in responsive hydrogel, graphene oxide, gold nanoparticle and QCM-based sensors [70-71]. Aptamer-based assays are divided into three classes depending on the signal-harvesting method; electrochemical, optical and mass-sensitive. Electrochemical assays include labelling aptamers with a redox-active component such as methylene blue, then immobilising it on an electrode to monitor the electron-transfer activity of the redox species. Optical assays include the use of either fluorophores, which carries the advantage of being able to provide real-time detection. As well as the fluorophore, the aptamer is conjugated directly with a quencher or a complementary DNA sequence labelled with a quencher, negating the fluorescence signal until it dissociates upon target binding [66]. Recent advances in the field have resulted in the incorporation of aptamers in optical, electrochemical and piezoelectric-based biosensors [5, 66, 72]. Aptamer-based biosensors have been developed for virus detection [73-74]. Figure 3, A-B, show examples of using aptamers for sensor developmnets for virus detection.

Molecularly imprinted polymers (MIPs) which are currently known as plastic antibodies are synthetic polymers which can be designed and synthesised to recognize and specifically bind to a target molecule [55, 77]. They are more resistant to chemical and biological damage and inactivation than antibodies [78]. A characteristic feature of MIPs is their hydrophobicity which allows for the weak reversible interactions between the target and the MIPs. Interacting species in the MIP can be assembled non-covalently, covalently or via ligand exchange. MIPs are designed to bind reversibly to their target. This is very important, as a MIP with too high an affinity will result in the target failing to dissociate,

or the MIP itself becoming damaged upon target removal. A crosslinking agent such as tetramethoxysilane is used to join the functional groups in place, creating the specific binding sites upon template removal [79]. Creating MIPs for small biomolecules is relatively straightforward as evidenced in the literature [80-82]. However creating MIPs for large biomolecules (e.g. proteins and viruses) remains a challenge [83]. Proteins are difficult to molecularly imprint because of the properties of their 3-D structures. These structures are flexible, complex and change quite easily with minimal energy required to adjust their conformations. Imprinting would prefer a more rigid type of structure [84]. Attempts to directly imprint proteins have been documented in scientific research however these attempts are limited to the cost of the generally expensive template proteins. A solution to this problem may be epitope imprinting. Epitope imprinting is the imprinting of only the epitopes (regions of molecules recognizable by affinity ligands) of the template protein to create specific cavities which recognise and selectively bind to the protein at these points. This is particularly useful when imprinting whole cells where membrane proteins can change their relative position in the membrane [83]. Epitopes in protein molecules are abundant, and data on epitopes can be easily accessed directly by crystallisation or indirectly through a database. Recently, even smaller epitope recognition sites – nanocavities – were implemented into hierarchically structured hydrogels to improve the selectivity and strength of MIPs targeted against proteins [85].

MIP technology still suffers from the fact that there is a lack of a standard manufacturing procedure. Special considerations must be taken into account when attempting to make a molecular imprint for viruses. Nanoparticles molecular imprinting can be used for this application. The uniformity of the pre-crosslinking mixture, as well as the release of the virus template after crosslinking, are two major factors affecting the formation of complementary cavities. Aggregation of the polymer reagent with the template must be prevented, as the resultant MIP will become complementary to the aggregate rather than the virus, and as a result increase the non-specific binding to the MIP, reducing its desired specificity. Washing the MIPs is also something that requires care, as it needs to be effective enough in removing the template from the hydrogel, but also not too strong as this may damage it. Therefore, a successful imprinting method must prevent aggregate formation before polymerisation as well as completely and efficiently remove virus templates from the MIP afterwards.

Several research groups have been able to produce MIPs nanoparticles for analyte imprinting [86]. The removal of viruses using MIPs has various applications in medicine, veterinary care, agriculture and biopharmaceuticals. Epitope-based MIPs targeted to bind the the glycoprotein 41 (gp41) transmembrane protein of HIV-1 were developed by Lu et al. [87], where it was found that the MIPs had a dissociation constant for the template similar to that of monoclonal antibodies, as well has having a high specificity to the target. More recent research by Wangchareansak et al. [88] involved the development of surface-imprinted polymers specific to the influenza H5N1 virus, and its analyte binding was tracked by a QCM sensor. There is a wide commercial interest for MIPs, particularly in solid phase extraction of samples and today there are few companies marketing these products such as Sigma-Aldrich's and POLYINTELL, France. MIPs are also attractive recognition element for biosensors and use for virus detection [89-90]. Figure 4, show an example of MIP used on biosensor surface [91].

4. Virus detection and viral biosensing systems

Conventional virus detection techniques such as virus isolation, immunofluorescence microscopy, polymerase chain reaction (PCR) and enzyme immunoassays are becoming obsolete as routine practices. High-throughput analysis of multiple samples has been widely carried out using enzyme-linked immunosorbent assays (ELISA) or enzyme-linked immunospotting (ELISPOT), but these are hampered by the fact that they can take around 3-5 hours to run, as well as a lack of sensitivity, giving false negative results under low virus concentrations. Therefore, present-day diagnostic tests depend solely on real-time PCR and more promising multiplex PCR techniques. Nucleic acid amplification techniques such as PCR and RT-PCR are currently the most-used viral detection methods, as they can gather important epidemiological information such as genotype data. This can then be implemented in vaccination programmes [10]. A specific challenge that is posed to molecular diagnostic techniques by waterborne viruses is that they have RNA rather than DNA genomes, which are more complex, dynamic and genetically variable. Multiplex PCR also saves time and resources as it amplifies and detects viral nucleic acids in a single step [92]. However, these are still expensive due to the need for specialist equipment and staff [93- 94]. PCR based methods also do not always correctly amplify viral nucleic acids when the viral concentration in samples is at a critically low level [95]. This will give inaccurate results for the analysis.

Biosensors are seen as a competent alternative to the above methods, aiming to modernise virus detection techniques. This is because they can be developed inexpensively into sensitive, specific tools that are easy to operate. They can also give very rapid readings, saving hours of sample analysis time compared to conventional methods [96-99]. Therefore, with all of these advantages, biosensor technology shows promise in becoming the future gold standard of virus diagnostics [93]. Along with this, the conventional molecular techniques carry a number of disadvantages. These include their incompatibility with virus concentration methods, their need for adequate sample volume, and their inability to distinguish infectious viruses [9]. As well as detection of a viral fragment rather than the whole unit makes it difficult to retrospectively link this to the number of virions present in the sample. In order to develop successful point-of-use biosensors, research must focus on detecting viruses in their actual environment with minimum sample preparation [6].

Biosensors are the fastest growing technology for the detection of viruses [94]. A biosensor is defined as an analytical device incorporating a molecular recognition element associated or integrated with a physiochemical transducers such as electrochemical, optical, mass sensitive, calorimetric or magnetic [55]. Biosensors require the selection of the optimal recognition elements (receptor) able to sensitively and selectively detect the target analyte of interest and then apply /immobilise the receptor on the surface of the transducer to enable a complete device. Antibodies, MIPs and aptamers are examples of the type of recognition receptors that can be used for virus recognition and detection. The targets are viral proteins, viral nucleic acids or antibodies produced by the host as a result of viral infection. Issues with bio-receptors are steric hindrance, denaturation and non-specific binding to non-target molecules can be a problem during the detection. Therefore successful incorporation of bio-receptors is critical to biosensor operation [49]. Before biosensors truly supersede current virus detection methods, there are still some hurdles that biosensor technologies need to overcome, such as the preparation of samples, system integration and sensitivity to sample matrix [100]. More advanced, real-time biosensors can be developed by implementing nanoparticles or smart materials, such as DNA which has been applied in clinical diagnostics to monitor patients on antiviral therapy for Hepatitis B & C, as well as HIV [101]. The biosensor's ease of use is critical especially for point- of - care devices. Therefore, a mass-produced biosensor design as a hand held device is favoured as it enables the analysis to be done on- site. Biosensor systems are foreseen to improve in tandem with the advances in nanotechnology, as this can lead to

smaller, finer tuned tools with the ability to analyse multiple, smaller samples in real-time. Sensors can be combined with microfluidics, which provides advantages such as small sample volumes, low-cost reagents, ease of fluid control, and ease of process automation [102]. Magnetic markers are becoming utilised more often in biosensor technology as their binding to biomolecules results in a change in electrical resistance, which can be detected by the sensor. Commercialization of biosensors technologies is a challenge but the *in vitro* diagnostics market is projected to grow at an annual rate of 25% in the immediate future [5]. The global demand for these devices will increase as countries become more concern with public health and food and environmental contamination.

An example of a sensing platform used for versus detection in research is a quartz crystal microbalance (QCM) sensor platform. It is a piezoelectric device, meaning that it detects electric charge accumulated in solid materials in response to mechanical stress. Specifically, a QCM sensor measures the change in mass of the gold-coated quartz crystal upon label-free analyte binding via a decrease in oscillating frequency, which is converted to a measurable signal [58, 97]. QCM sensors are able to quantify a bound analyte, and there are already several examples where this has been applied in virus detection [103-105]. There are a number of advantages to using QCM biosensors, such as ease of use, cost-effectiveness, high sensitivity and ability to quantify an analyte in real time. As well as mass changes of the crystal, the QCM also picks up surface properties such as density, viscosity, temperature and pressure, so it is important that these variables are controlled [97, 104]. Signals can also be affected by factors such as the roughness of the surface electrode, shear oscillation amplitude, immersion configuration and hydrostatic pressure in a flow system. QCM sensors are versatile in that they can sense particles between 100-1000 nm in size, meaning that they can be tailored to detect a variety of virus particles. Because of its recent developments such as its quantification range and improved sensitivity, its abilities equal or even surpass those of more conventional optical procedures [72].

Surface plasmon resonance (SPR) spectroscopy is a very sensitive, fast diagnostic technique that uses the indirect detection of surface plasmons from absorption features that correspond to the coupling of light to the surface plasmon [106]. Surface plasmons are electromagnetic waves that oscillate at the interface of two media, such as a metal and dielectric that have opposite-sign permittivity. The recognition element are immobilised onto the metal surface, such as gold or silver [107]. SPR biosensors are growing in popularity and confidence among researchers as

tools for medical diagnosis and biomolecular analysis, as they can provide detailed information on the affinity, specificity, analyte concentration levels and kinetics of binding interactions in real-time and label-free.

SPR is sensitive to changes in the refractive index of the dielectric upon analyte binding to the substrate-metal interface as it passes through a fluidic channel [108]. There are three methods of surface plasmon excitation; prism-based configuration, waveguide-based approaches, or by using an optical fibre-based architecture. Using a prism-based configuration, resonance is monitored by collecting the reflected light as a function of the incidence angle at a fixed wavelength, or as a function of the incident wavelength at a fixed angle. In waveguide or optical-fibre based methods, surface plasmons are excited by a broadband light source, and the transmitted light is collected and analysed, where a dip in the spectrum represents a change in resonance [107].

Electrochemical biosensors have been developed for virus detection as portable, simple, easy to use and cost effective when combined with a small handheld instrument. The sensor can be used for on-site analysis that can be conducted by non-expert personnel [52]. Many of these sensors are based on amperometric transducers and a screen-printed disposable electrode. The type reported for viruses analysis is based on affinity principle where the antibody is used to bind to the virus either in a capture for impedance detection system or as sandwich assay as the amperometery detection system with an enzyme label [109].

Viral infections can progress and spread very quickly, meaning that conventional detection methods are unfavourable due to their time-consuming nature. In order to provide an adequate response to contamination of water such as its remediation, as well as the swift treatment of infected individuals, rapid, specific and selective diagnosis is a must. Virus concentration from samples may be required before the sample can be analysed. To date there are diverse methods based on adsorption-elution (charged filters), perspiration (organic flocculation with ammonium sulphate or polyethylene glycol), ultracentrifugation, lyophilisation, ultrafiltration, and magnetic beads affinity separation [10] which are used for this application The rapidly-growing diagnostics market now emphasises the generation of these [110]. Aptamers, antibodies and MIPs can be utilised as recognition elements in viral biosensors, as these can be engineered to target viral protein/nucleic acids as well as antibodies

resulting from an immune response. This can be achieved either by physioadsorption onto conducting polymer surfaces such as polyaniline/polypyrrole, or onto a monolayer by covalently coupling them to a linker via amino, maleimido, carboxyl or thiol groups bound to transducer surfaces such as carbon, silicon, gold or a hydrogel. Very recently, a label-free diagnostic method of viruses was demonstrated that measured the orientation behaviour of liquid crystals on polymer-treated gold surface upon virus binding [111]. Biosensors as a cheaper and faster diagnostic tool for waterborne viruses in particular would be welcomed as the annual worldwide costs related to waterborne diseases are in the billions. This is not helped by fast-spreading outbreaks – in 2008, around €870,000 worth of medical expenses were generated by a single norovirus outbreak in the Swedish municipality of Lilla Edet alone [112] – hence, quick, early-stage detection methods can reduce these costs. Table 3, summarises an example of biosensors developed detection of viruses.

The applications of nanomaterials such as quantum dots, carbon nanotubes and smart nanostructures for the detection of viruses using biosensors also play an important role to increase the sensitivity and capacity of these platforms. Liu et al developed a graphene based electrochemical biosensor for highly sensitive pathogenic virus detection. A 10^5 pfu.mL^{-1} of input cells is detected with ca. 30.7% sensitivity, and ca. 1.3% sensitivity is measured with 10^3 pfu.mL^{-1} of input cells [120]. In another study, avian influenza virus was detected by an electrochemical sensor using a DNA aptamer immobilized onto a hybrid nanomaterial-modified electrode. To enhance the selectivity and sensitivity, the modified sensor was assembled with multi-wall carbon nanotubes (MWNT), polypyrrole nanowires (PPNWs) and gold nanoparticles (GNPs). This electrode provided a porous structure with a large effective surface area, highly electrocatalytic activities and electronic conductivity. The researchers achieved the detection limit of 4.3×10^{-13} M and confirmed that the new hybrid nanomaterial (MWNT/PPNWs/GNPs) and the DNA aptamer could be used to fabricate an electrochemical biosensor for virus detection [121]. Lee et al developed a plasmon-assisted fluoro-immunoassay (PAFI) for the detection of the influenza virus by using gold nanoparticle (Au NP)-decorated carbon nanotubes (AuCNTs). Specific antibodies against the influenza virus were conjugated onto the surface of AuCNTs and cadmium telluride quantum dots (QDs), which had a photoluminescence intensity that varied as a function of virus concentration and a detection limit of 0.1 pg.mL^{-1} for three different types of influenza viruses were achieved. The clinically isolated influenza viruses were detected in the range of 50–

10,000 pfu.mL^{-1}, with a detection limit of 50 pfu.mL^{-1}. The system provided not only robust signal production and amplification, but also an excellent sensitivity and selectivity for influenza viruses [122]. The application of this work can be extended for the detection of many other viruses. More examples for nanomaterials application in the area of virus detection employing biosensors can be found from current literature [123-125].

Conclusion

The annual direct medical related expenses for waterborne diseases are in the billions of dollars range making them very expense and of serious concern to public health institutions. Therefore, virus filtration and detection methods are very important because they can reduce the cost of these medical expenses by quickly investigating viral outbreaks and aiding in appropriate prevention and treatment strategies. The development of more specific removal systems and sensors can efficiently identify and quantify the presence of a virus in water samples in an easy, quick, sensitive and inexpensive manner. Advances in the field of bio-nanotechnology will result in the improvement of sensors and sensor systems to be smaller, more sensitive, with the ability for real time, multi-analyte detection requiring smaller quantities of sample volumes.

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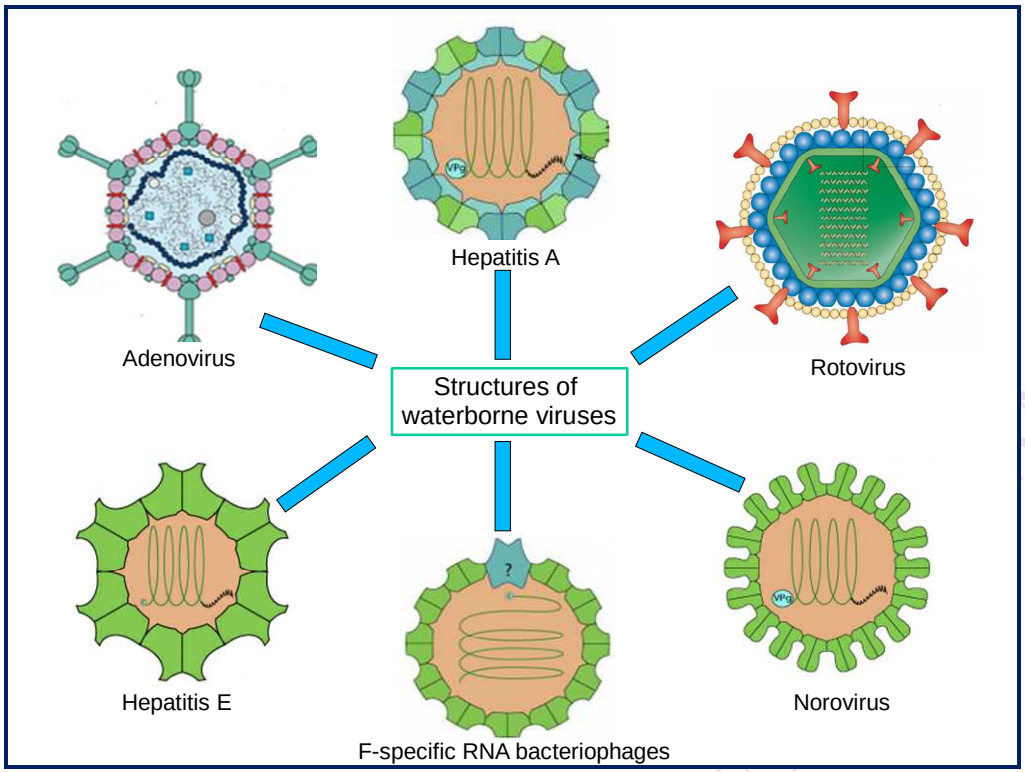
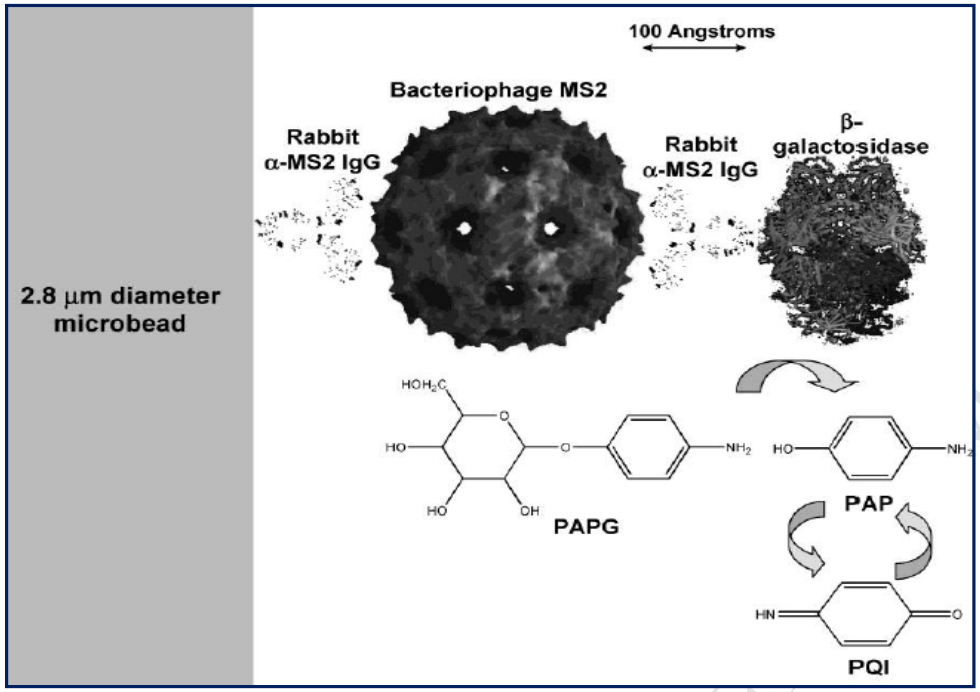


Figure 2

(A)



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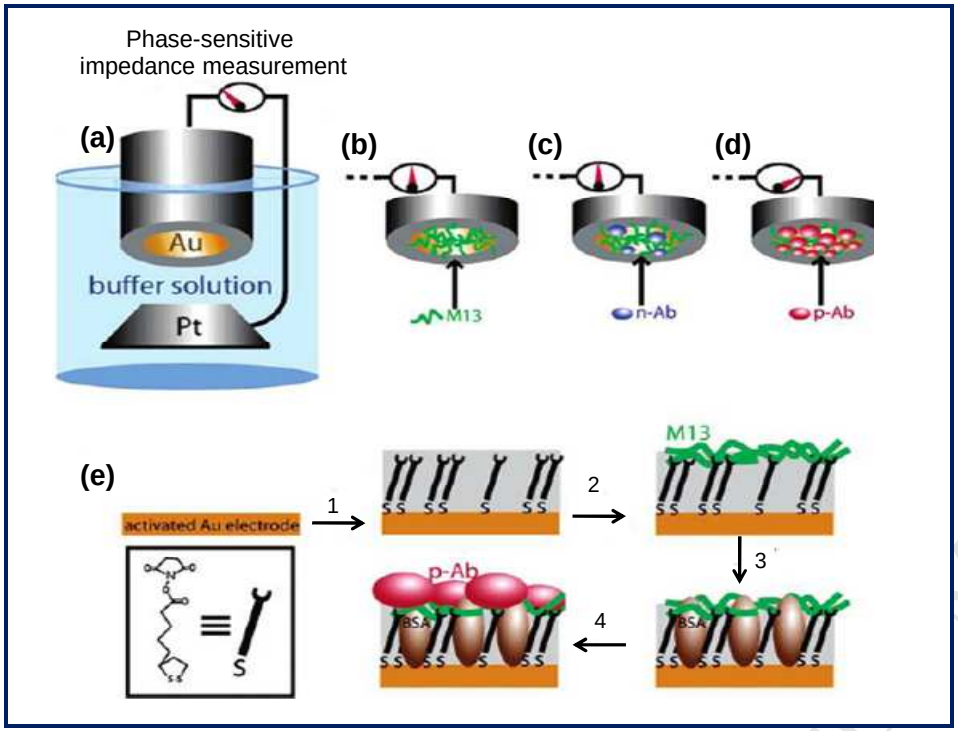
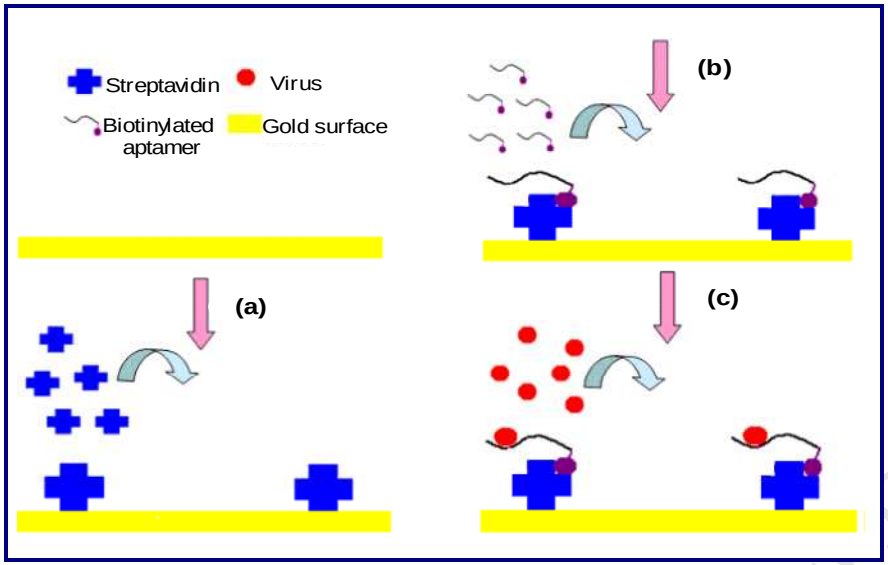


Figure 3

(A)



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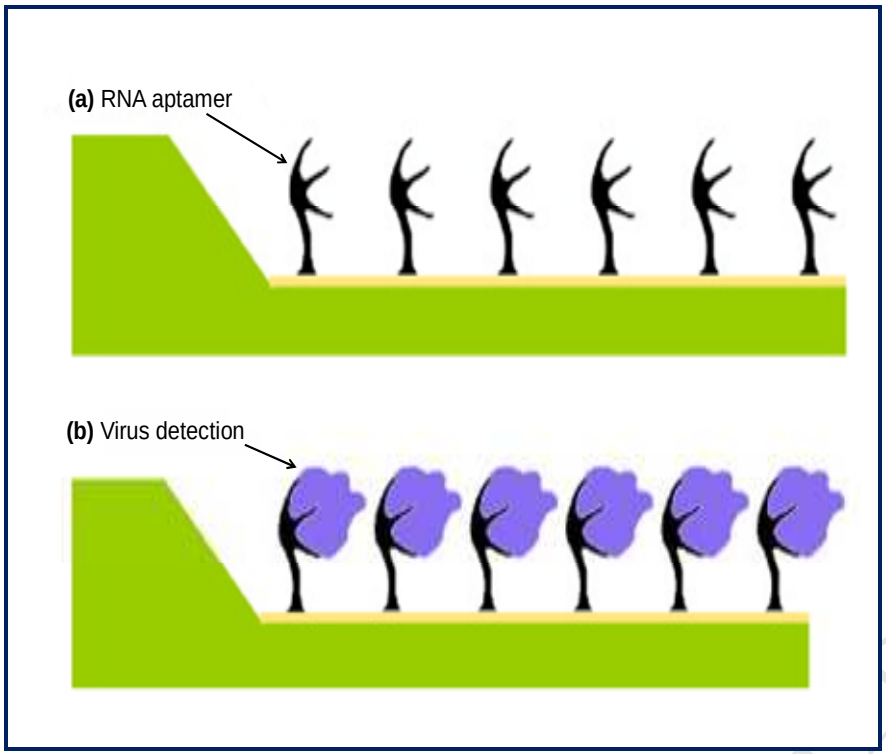

Figure 4

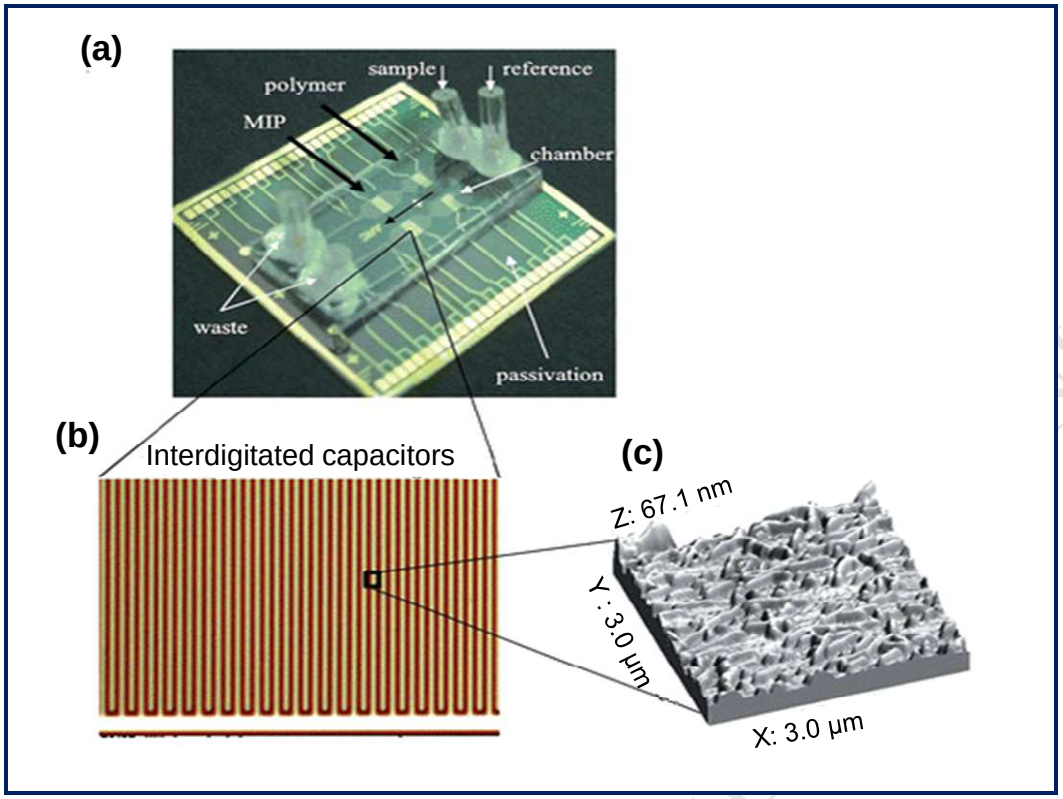

Table 1

Virus	Size (nm)	Genome	Shape	Available vaccine	Symptoms & Diseases	Reference
Adenovirus	80-90	Double-stranded DNA molecule	Icosahedral	No vaccine	Respiratory, ocular, and enteric infections Pneumonia, Genitourinary diseases and gastroenteritis	[12-13, 116]

Hepatitis A	27	Positive-sense, poly-adenylated RNA molecule	Icosahedral	Yes, vaccination is generally effective	Infection causes fever, jaundice, nausea, fatigue, abdominal cramps and anorexia	[3, 13, 117]
Hepatitis E	27-34	Positive-sense, poly-adenylated, and single-stranded RNA molecule	Icosahedral	Available vaccine but needs to be evaluated for safety and efficacy	Inflammation of the liver which results in incapacitating pain, vomiting and fever Incapacitation of the patient	[12, 117]
Norovirus	27-32	Positive sense, single-stranded RNA molecule	Spherical	No regulated vaccine; potential vaccines in phase I/II clinical trials	Vomiting, diarrhoea, nausea and abdominal pain, Dehydration, Gastroenteritis	[117]
Rotovirus	75	Double stranded RNA molecule	Icosahedral	Yes, but the variability of the different groups of viruses can make successful vaccination difficult	Diarrhoea, dehydration, vomiting and fever Gastroenteritis	[3, 12, 117-118]
Bacteriophage MS2 (F-specific RNA viruses)	22.4–28.8	Single stranded RNA molecule	Icosahedral	No, not harmful to humans	N/A	[119]
Bacteriophage GA (F-specific RNA viruses)	22.7–28.9	Single stranded RNA molecule	Icosahedral	No, not harmful to humans	N/A	[17]
Bacteriophage Qβ (F-specific RNA viruses)	21.3–29.4	Single stranded RNA molecule	Icosahedral	No, not harmful to humans	N/A	[17]
Bacteriophage SP (F-specific RNA viruses)	26	Single stranded RNA molecule	Icosahedral	No, not harmful to humans	N/A	[17]

Table 2

Company Name	**Product Name**	**Filtration Basis (Tangential/Normal Flow)**	**Viruses Targeted**

Millipore	NFP	Normal	>4 log ΦX-174 bacteriophage
	NFR	Normal	>6 log retrovirus
	Viresolve 70	Tangential	>4 log polio >7 log retrovirus
	Viresolve 180	Tangential	>3 log polio >6 log retrovirus
Sartorius	Virosart CPV	Normal	>4 log PP7 bacteriophage >6 log retrovirus
Pall	DV20	Normal	>3 log PP7 bacteriophage >6 log PR772 bacteriophage
	DV50	Normal	>6 log PR772 bacteriophage
Asahi	Planova 15N	Normal/Tangential	>6.2 log parvovirus >6.7 log poliovirus
	Planova 20N	Normal/Tangential	>4.3 log parvovirus >5.4 log Encephalomyocarditis
	Planova 35N	Normal/Tangential	>5.9 log Bovine viral diarrhoea virus, >7.3 log HIV

Table 3

Sensor Type	Detection Principle	Detected Analyte	Limit of Detection	Reference
Piezoelectric based-QCM sensors	Detects the binding event which occurs when the analyte binds to the sensor's electrode and changes the frequency of the quartz resonator	M13 M13 Influenza A	20 phages 10^6 pfu.mL^{-1} 4 virus particles.mL^{-1}	[49-72, 109]
Optical based-SPR sensors	Indirect, inferred detection of the existence of surface plasmons from the resultant absorption by the analyte which corresponds to the coupling of light to the surface plasmon	Hepatitis C Hepatitis B Infectious bursal disease virus	60 pM 2 fg.mL^{-1} 2.5 ng.mL^{-1}	[49, 94, 113, 106]
Electrochemical-based sensors	Direct conversion of a biological event (analyte binding to affinity ligand) to an electronic signal	Bacteriophage MS2 Bacteriophage MS2 Hepatitis B Adenovirus Rotavirus	3.2 x 10^{10} viral particles.mL^{-1} 1.5 x 10^{10} viral particles.mL^{-1} 7.19×10^{-9} M 10^3 virus particles.mL^{-1} 10^3 pfu.mL^{-1}	[14, 49, 64,109, 114,115]

Highlights

- Waterborne viruses and importance of water quality for human health is described.
- Seperation and filtration techiques for virus removal from water is reported.
- Affinity-recognition of virus has great potential with natural and artifical ligands.
- Sensor technology provides a strong alternative to other detection techniques.
- Different kind of biosensors can be used for waterborne virus detection and removal.