Detection of viruses with molecularly imprinted polymers integrated on a microfluidic biochip using contact-less dielectric microsensors†

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Rapid detection of viral contamination remains a pressing issue in various fields related to human health including clinical diagnostics, the monitoring of food-borne pathogens, the detection of biological warfare agents as well as in viral clearance studies for biopharmaceutical products.

The majority of currently available assays for virus detection are expensive, time-consuming, and labor-intensive. In the present work we report the creation of a novel micro total analysis system (µTAS) capable of continuously monitoring viral contamination with high sensitivity and selectivity. The specific interaction between shape and surface chemistry between molecular imprinted polymer (MIP) and virus resulted in the elimination of non-specific interaction in the present sensor configuration. The additional integration of the blank (non-imprinted) polymer further allowed for the identification of non-specific adsorption events. The novel combination of microfluidics containing integrated native polymer and MIP with contact-less dielectric microsensors is evaluated using the Tobacco Mosaic Virus (TMV) and the Human Rhinovirus serotype 2 (HRV2). Results show that viral binding and dissociation events can be readily detected using contact-less bioimpedance spectroscopy optimized for specific frequencies. In the present study optimum sensor performance was achieved at 203 kHz within the applied frequency range of 5–500 kHz. Complete removal of the virus from the MIP and device reusability is successfully demonstrated following a 50-fold increase in fluid velocity.

Evaluation of the microfluidic biochip revealed that microchip technology is ideally suited to detect a broader range of viral contaminations with high sensitivity by selectively adjusting microfluidic conditions, sensor geometries and choice of MIP polymeric material.

Introduction

The incidence of viral distribution and infection has soared over the past decades due to population growth and increased travel.1 As a consequence, rapid detection and identification of viral contamination is a pressing human health issue in fields ranging from clinical diagnostics and monitoring of food-borne pathogens to the detection of biological warfare agents. Faster, more accurate diagnosis will benefit health care systems in particular, by significantly reducing costs and providing better epidemiological data that can be used for infectious disease modeling.2

Another benefit of improved viral diagnostic methods relates to the improved screening of biotechnology associated products that may pose a risk to human health. For instance, the majority of mammalian cell lines used to produce biopharmaceutical products harbor viruses that may in some instances infect human cells. The risk of cross-infection is significant enough for European Union Regulatory Compliance to stipulate that pharmaceutical companies demonstrate the absence of retrovirus infectivity through extensive testing procedures.3

The majority of currently available assays to detect viral contamination are expensive, time-consuming, and labor-intensive. A variety of nucleic acid-based assays and immunologic techniques have been developed to detect viruses, including real-time PCR, ELISA, electron microscopy or titration in indicator cells (e.g. S+L-cell lines) which display a transformed phenotype following retrovirus infection.4,5 Despite the variety of biosensors and bioassays developed in the past decade to advance the diagnostic field, there is still a need for miniaturized, low-cost or disposable biosensors capable of rapidly detecting and accurately identifying a wide range of pathogens.6–9 One prominent example of a low-cost (1/4 $ per unit) and rapid (minutes) HIV screening method is based on dipstick technology.9 Here, immunochromatographic strips that incorporate both antigens and signal reagent into a nitrocellulose membrane are used to differentiate between HIV-1 groups M, O and HIV-2 serotype, respectively. Additionally, the area of miniaturized or microfluidic analysis systems, also called ‘micro total analysis systems (µTAS) or lab-on-a-chip (LOC), has gained increased popularity for the detection of pathogens.10–12 The ability of microfluidic systems to conduct measurements from small volumes of complex fluids
with efficiency and speed, without the need for a skilled operator, has been regarded as the most powerful application of LOC technology.\(^2\) In recent years, the microchip analysis of conserved DNA or RNA sequences using PCR and RT-PCR techniques has been extensively used to detect infectious diseases and determine the actual stage of the disease.\(^3\) Another powerful analytical tool for pathogen detection in a microfluidic biochip employs immunological methods that rely on the specific affinities of protein–protein, protein–carbohydrate or protein–DNA interactions.\(^4\) Here Antigen (Ag)/antibody (Ab) recognition systems are widely accepted for pathogen detection.\(^5,17\) Although antibodies, polyclonal Abs (pAb), monoclonal Abs (mAb), recombinant antibody-fragments (rAbs) or single chain variable fragments (scFv) can be readily obtained. A major drawback of immobilized antibodies includes poor binding-site recognition following sensor surface functionalization, leading to decreased sensitivity and reusability.\(^18\) In other words, antibody-based pathogen recognition systems need to preserve the native protein state for optimal orientation of the protein–target interaction after immobilization.\(^21\)–\(^23\)

The application of molecular imprinted polymers (MIPs) as the sensing layer offers a real alternative to antibodies due to their inherent robustness and reproducibility.\(^24\)–\(^25\) MIPs were originally developed in the 1970s\(^25\)–\(^26\) and provide three-dimensional and highly specific receptor sites by forming a polymeric matrix in the presence of target analytes. Following template removal, vacated cavities with complementary size, shape and orientation of the analyte are obtained. Over the last decade, MIPs have been extensively used to detect small and large molecules, proteins and even microorganisms, such as bacteria and yeast.\(^27\)–\(^32\) Additionally, MIPs are capable of mimicking the recognition and binding capabilities of natural biomolecules and have therefore been employed to remove, purify and detect viral contamination.\(^33\) The fabrication of MIP systems for viral detection involves the assembly of a two-dimensional surface imprinting ‘stamp’ containing the virus targets brought into contact with a cross-linked polymer that is attached to the sensor surface. Also, a variety of analytical detection methods have been successfully coupled with MIP systems, including optical, electrochemical and acoustic (SAW) detectors.\(^7\)–\(^34\)–\(^35\) However, surface plasmon resonance (SPR) and mass-sensitive sensors, such as quartz crystal microbalances (QCM), are probably the most popular due to their selectivity and sensitivity.\(^36\)–\(^38\) Despite the versatility of MIP systems, they have not been widely employed in combination with microfluidic devices or \(\mu\)TAS. The only study to date that has integrated MIPs with microfluidics involved the simultaneous detection of cholesterol, progesterone and testosterone using SPR.\(^39\)

In the present work we report the development of a microfluidic biochip containing integrated MIPs to detect viral contamination using contact-less dielectric microsensors. In contrast to the application of MIPs in capillary electrophoresis,\(^40\)–\(^41\) where affinity binding is used to support separation over a much longer channel, the setup presented here resembles a sensor system with only one theoretical plate for separation. Therefore, selectivity has to be substantially increased to achieve the necessary recognition ability in the system. Dielectric spectroscopy is generally used to measure the electrical properties of polarization responses of low dielectric materials such as (bio)polymers, proteins or cells exposed to radio-frequency electrical fields.\(^42\)–\(^44\) A number of recent studies have also shown that the electric properties of viruses can be used to manipulate, detect and identify them using dielectric spectroscopy, dielectrophoresis and AC capacitance, respectively.\(^45\)–\(^47\)

In our previous work we have shown that the application of high-density interdigitated capacitors (\(\mu\)IDCs) eliminates common interferences of bioimpedance spectroscopy such as pH, salt and protein content variations and are ideally suited to analyze responses of living cells to external stimuli.\(^48\) In contrast to existing impedance methods, the contact-less microsensors (\(\mu\)IDCs) are completely insulated and physically removed from the liquid sensing environment, thus providing stable and non-drifting measurement conditions.\(^48\) Consequently, the application of microfabricated interdigitated capacitors continuously monitors changes in the intrinsic dielectric properties of imprinted polymers (MIPs) in the presence of viral binding events leading to reorientation of dielectric dipoles and alterations in the surface polarization.\(^49\) In the present work we have developed, characterized and evaluated a microfluidic biochip consisting of reaction and reference channelshousing the four contact-less dielectric microsensors covered with either native- or imprinted-polymers (MIP). The creation of a low noise detection environment eliminates background effects and interferences (e.g. unspecific adsorption), thus allowing simultaneous detection of viral binding and dissociation events. In the present study the newly developed microfluidic biochip is applied for the high-sensitivity detection and identification of viral contamination using Tobacco Mosaic Virus (TMV) and Human Rhinovirus serotype 2 (HRV) as models.

**Experimental**

**MIP chip design and fabrication**

The lab-on-a-chip system consists of the biochip located in an aluminium block, external valves and pumping stations (ESIt). The microfluidic biochip was fixed using spring loaded connectors embedded within two printed circuit boards providing shielded connection to the potentiostat (VMP3, Bio-Logic). The microfluidic biochip shown in Fig. 1A consisted of a 30 mm × 30 mm quartz glass substrate (Borofloat\(^\circledR\)) containing the electronic sensors, and a 15 mm × 25 mm polymer (PDMS) fluidic sandwiched between a microfluidic interface (pre-drilled glass substrate) connecting the microchannels to external fluidic reservoirs using a syringe pump (KDS 250, KdScientific). The fabrication of electrodes commenced by spinning the non-photo-sensitive resist (LOR 3A, MicroChem\(^\circledR\)) on the wafer at 3,000 rpm for 35 s. A second layer using photo resist (positive resist AZ\(^\circledR\)MIRTM 701) was applied and soft baked at 110 °C, 60 s. The resist was patterned using an MJB 3 mask aligner from SUSS Microtec (350 W mercury lamp, exposure time 6 s) and developed for 25 s (AZ\(^\circledR\)MIF 726). Electrodes, leads, as well as contact pads, were fabricated by sputtering first 10 nm titanium seed layer followed by 150 nm gold onto the pre-patterned substrates. The interdigitated electrode structures (\(\mu\)IDCs) shown in Fig. 1B were further covered by a 400 nm silicon nitrate and 50 nm silicon oxide bi-layer deposited via low temperature plasma enhanced chemical vapour deposition (PECVD, Oxford Plasmalab 80).
Next, the integration of MIP as seen in Fig. 1C was accomplished by pressing the virus stamp into the co-polymer of vinylpyrrolidone and methacrylic acid spin-coated with a layer height of 200 nm on the device. Polymerization was first started by adding 1.5 mg 2,2'-azobis-isobutyronitrile (70 °C) to an aqueous solution containing 30 mg N,N’-(1,2-dihydroxy-ethylene)bis-acrylamide (DHEBA), 20 mg 1-vinyl-2-pyrrolidinone and 50 mg methacrylic acid. The Tobacco Mosaic Virus (TMV) stamp was constructed by placing 10 μL of a 1.09 mg/mL TMV solution onto a 10 mm × 5 mm glass substrate (roughness approximately 2 nm; Schott, Mainz, Germany). Virus particle assembly was allowed to commence for 30 min at 4 °C. At this point the remaining fluid was removed and the stamp was clamped into the pre-polymer by a standard paper clamp and molecular imprinting took place for 16 hours at 4 °C. As both the stamp and the IDC on the substrate are some millimetres in length and width, alignment between the stamp and the surface to be imprinted can be achieved visually without further equipment or alignment tools. After removing the stamp substrate and washing the surface, the presence of the MIP layer could be seen by impedance changes. Concurrently, the soft polymer (PDMS) microfluidic was cast using a silicon master mold, which was fabricated by KOH etching using a 750 nm thick silicon oxide layer as hard mask. After stripping the photo resist (MIR 701), the substrate was etched in a 40% KOH solution at a constant temperature of 80 °C for 60 min resulting in an etch depth of 48 μm. Reaction chambers and channels were formed by pouring a PDMS (10:1 Sylgard 184) silicone elastomer base to a curing agent over the silicon master. After baking at 80 °C for 6 hours the PDMS fluidic was removed from the mold, cleaned and exposed to a 70 s treatment using a high frequency plasma discharger generator (BD-20V, Electro-Technic Products, Inc.) prior to bonding. Immediately after PDMS activation the PDMS fluidic was aligned under a microscope and put into contact with the sensor substrate. Final chip assembly involved the covalent attachment of the pre-drilled glass cover interface containing various self-made nanoports.

**Preparation of viral samples**

*Nicotiana tabacum*, the host plant, was systemically infected with TMV-U1, cultivated and the ground vein-free leaves (100 g) were stored in liquid nitrogen prior to TMV purification and isolation. A detailed description of the TMV purification and isolation procedure can be found elsewhere. The dried powder was resuspended in 200 mL Na2HPO4/KH2PO4 (100 mmol/L) containing 200 μL 2-mercaptoethanol (Merck). The suspension was filtered using cheese cloth and 15 mL butanol (Merk) was added to the filtrate and stirred for 15 min at 4 °C. Next the crude filtrate was centrifuged at 10 000 rpm for 10 min at 4 °C using SS34-rotor Sorvall-centrifuge (type RC 5C Plus). The supernatant was removed and 8 g NaCl and 6 g poly(ethylene glycol) (PEG 6000, Merck) were added to the suspension. Precipitation was allowed to commence for 15 min followed by centrifugation. The supernatant was discarded and the pellet was re-suspended in 16 mL KH2PO4/K2HPO4 buffer (10 mmol/L at pH 7.0) containing 800 μL 100% Triton X-100. Following an additional centrifugation step at 41 000 rpm (288 000 g) for 1 h at 4 °C (Beckmann ultracentrifuge, type L-70) using a sucrose pad (buffered 25% sucrose) the pellet was washed three times using 3 mL of a 10 mmol/L KH2PO4/K2HPO4 buffer (pH 7.0) and centrifuged until the required purity was obtained. In summary, approximately 1 mg (10 ppm) of purified TMV was obtained from 100 g *N. tabacum* leaves. 200 μL aliquots of the buffered virus sample were frozen using liquid nitrogen and stored at −80 °C until use. The TMV samples were characterized using phase image atomic force microscopy (AFM) and viral concentrations of samples were determined by UV-spectroscopy.

Preparation and purification of Human Rhinovirus serotype 2 (HRV2) is described in more detail elsewhere. HRV2 was grown in Rhino-HeLa cells in suspension culture and purified as described by Skern et al. (1984) with minor modifications. Briefly, following 16 h after infection cells were ruptured using three freezing and thawing cycles. Cell debris was removed by centrifugation for 45 min at 20 000 rpm in an SS34 Sorvall rotor. The virus-containing supernatant was removed and pelleted at 30 000 rpm for 2 h in a Beckman Ti45 fixed angle rotor. The viral pellet was suspended in 1 mL of a 20 mM Tris/HCl buffer (pH 7.4) containing 2 mM MgCl2, where contaminants were digested for 10 min at room temperature using RNase A and DNase, 50 mg/mL. Next 1.5 mg/mL trypsin was added to the suspension and incubated for 5 min at 37 °C. After addition of 0.3% in N-laurylsarcosine the suspension was stored overnight at 4 °C and insoluble material was removed by centrifugation for 15 min in an Eppendorf centrifuge. Virus purification was accomplished via sucrose density gradient centrifugation. The virus band was aspirated, diluted and pelleted. The purified HRV2 sample was suspended in 50 mM Tris/HCl (pH 7.4) and the concentration was determined using capillary electrophoresis. 100 μL aliquots containing 3 mg/mL HRV2 were stored at −80 °C prior to use.
Impedance measurements using contact-less dielectric microsensors

At first, passivation quality and complete insulation of the interdigitated capacitors (µIDCs) was confirmed using cyclic voltammetry (CV). The absence of ohmic currents and faradaic contributions in the presence of 5 mmol/L K₄[Fe(CN)₆] and 5 mmol/L K₃[Fe(CN)₆], in 1 M KCl solution was assessed using a two-electrode setup employing the interdigitated electrode structures as working and an external silver wire as quasi-reference electrodes (AgQREF). Observed currents above 10 nA were indicative of the presence of pin holes and passivation failures (see Fig. 2A).

Following sensor characterization, impedance measurements were conducted over a frequency range of 5–500 kHz in the presence of an applied peak-to-peak voltage (Vpp) of 200 mV using a multichannel potentiostat (VMP3/P-01, Princeton Applied Research). Unless otherwise noted, 100 µL of virus- and buffer-containing syringes were loaded into the pump and connected via PEEK (ID 0.13 mm) tubing to the fluid ports of the microfluidic biochip. The four dielectric microsensors (µIDCs) were addressed simultaneously by continuously recording absolute impedance (|Z|) and phase angle values (θ) of 51 frequencies.

Simulation of electric field distributions of contact-less dielectric microsensors

One benefit of using interdigitated electrode structures is the ability to tune the electric field distribution (H) in the z-direction by controlling the ratio between the spacing (G) and width (W) of the individual fingers (H = W + G). Using the conformal mapping technique, electric field distributions were calculated using interdigitated electrode structures consisting of 200 fingers (5 × 5 × 1000 µm width, gap and length). In the present configuration 90% of the electric field was found to protrude to approx. 2.4 µm in the z-direction while two-thirds of the applied potential dropped off within the 400 nm SiN/SiO₂ passivation layer (see ESI†).

Chemometric data analysis

Dielectric spectroscopy data obtained for three replicate runs with each sample were used to generate the matrix for principal component analysis (PCA). Each column in the data matrix consists of 51 signals obtained for buffer, TMV virus sample and water in the presence and absence of MIPs. The matrix was converted into a text file for incorporation into MATLAB (Version 7.01). Factor analysis was performed using the Chemometric Toolbox for Matlab (Version 3.02), and involved the generation of reduced eigenvectors, examination of the resulting residual plots for randomness of noise, and the generation of scores for the first three principal components. The obtained scores were plotted to determine qualitative groupings of individual data sets.  

Results and discussion

MIP chip characterization

The aim of this research is the development and characterization of a novel technology capable of continuously monitoring viral contaminations with high sensitivity using molecular imprinted polymers (MIPs) as sensing elements. An important feature of the newly developed detection platform is the ability to monitor capacitive changes of the MIP using contact-less dielectric microsensors. In a first set of experiments, the effects of sensor isolation were evaluated in the presence of MIPs. Fig. 2A shows cyclic voltammograms of MIP-covered dielectric sensors in the presence of 5 mM ferrocyanide. In the absence of sensor passivation (ii), the 200 nm thick MIP layer allows significant charge transfer reactions to occur. In turn, Fig. 2B showed a 300-fold higher imaginary part and a 20-fold higher real part of the impedance values in the presence of ca. 400 nm thick SiN/SiO₂ passivation. In other words, sensor isolation and physical removal from the liquid sensing environment eliminates ohmic contributions, thus significantly increasing absolute impedance values. Since the capacitive contributions dominate the overall signal (IXC ≫ R), the classic impedance equation Z = R + jXC can be simplified to Z ≈ jXC or 1/jωC. This means that contact-less impedance measurements are particularly sensitive towards capacitive changes occurring along the surface of the MIP. As a result, MIP stability under fluid mechanical shear force conditions is an important issue to consider. Stability tests conducted over a period of 20 hours in the presence of 100 mM...
phosphate buffer perfused at 35 μm/s showed small signal decreases during the initial two hours followed by stable and non-drifting background signals. Observed impedance changes are indicative of polymer swelling events that take place during the early stages of buffer perfusion (see ESI†). If not otherwise stated, the microfluidic biochip was rinsed with buffer for a period of three hours prior to each measurement.

**Assay optimization and frequency analysis**

The underlying principle of the presented detection method is based on intrinsic dielectric differences between the polymer used to fabricate the MIP and the virus sample. Consequently, the ability of the contact-less dielectric microsensors to distinguish between the electric properties of TMV and the applied polymeric material, poly(methacrylic acid-co-vinylpyrrolidone), was initially investigated. Here, PDMS containing four openings was layered above the dielectric microsensors featuring four wells of approx. 30 μL. After 5 hours of pre-incubation, the volumes above the two MIPs and the two contact-less dielectric microsensors covered with the non-imprint polymer were exchanged with de-ionized water (DI), buffer and 4 mg/mL TMV solutions. Following 45 min incubation at RT, three replicate measurements were conducted and resulting dielectric spectra were used to generate a 101 × 18 data matrix. The matrix was converted into a text file that was imported into MATLAB (Version 7.01) to perform Factor Analysis using the Chemometric Toolbox (Version 3.02). Fig. 3 shows the results of principal component analysis (PCA) for data points obtained in the presence of DI, buffer and virus solution using molecular imprinted and native polymers. Results clearly indicate that dielectric spectroscopy is not only able to distinguish between different analytes, but can also detect intrinsic structural differences between molecular imprinted- and native-polymers. For instance, as seen in Fig. 3, TMV-containing samples (▲, △) are clustered in close proximity, but are grouped sufficiently apart to allow discrimination between native and imprinted polymer. As is common in PCA, the components themselves (PC1 and PC2) do not have immediate physical meaning but rather denote new, linearly independent coordinate axes for multivariate data clusters. However, they give a good indication of how individual points are grouped within the data set. In a second set of experiments (data not shown) three wash steps were found to be sufficient to remove unspecific TMV adsorption. In other words, precise control over flow profiles within the microfluidic biochip may completely eliminate unspecific adsorption while maintaining optimum binding conditions for the TMV virion.

Subsequent experiments employed microfluidics and were conducted using a 100-fold dilution or 40 μg/mL TMV solution in the presence of 35 μm/s fluid flow. Fig. 4A shows four impedance traces of the microfluidic biochip obtained for 1 hour following TMV injection. Significant impedance increases were found for both native- and imprinted-polymers in the presence of TMV, while references showed stable background signals. However, only in the presence of the MIP do impedance signals clearly remain above the initial background indicating viral binding events. The overall decrease in dielectric permittivity in the presence of virus particles leads to the observed impedance signal increase. It is also important to note that, in the course of an experiment, impedance values over 51 frequencies are recorded simultaneously from each contact-less dielectric...
microsensor. In an attempt to identify optimum frequency ranges, the percent impedance differences (ΔIZI) prior to and after virus injection were calculated for the MIP-covered dielectric sensor. Fig. 4B shows a comparison of selected frequencies exhibiting a distinct frequency dependent maximum at around 203 kHz using TMV solution.

**On-chip monitoring of viral contaminations**

To further investigate robustness, reusability and sensitivity of the molecular imprinted (MIP) lab-on-a-chip system, viral binding affinities under increased shear force conditions and sample dilutions were tested. Initially, both microfluidic arms were simultaneously perfused with buffer solutions at a flow velocity of approx. 35 μm/s. Fig. 5A shows impedance time traces obtained over a period of 24 hours. The initial virus peak obtained during 1 hour exposure to 8 μg/mL TMV is clearly visible in the sample chamber (ii), while no signal changes were found in the reference arm (i). However, in the presence of a 50-fold increase of fluid flow from 35 to 1750 μm/s, impedance signals decreased from the elevated plateau level to background signals. The signal loss in the presence of increased shear force conditions indicates virus detachment and removal from the MIP cavities. Since both reaction and reference chambers exhibited similar signal drifts over a period of 10 hours, reference signals were further subtracted to eliminate background noise. Fig. 5B shows normalized impedance time traces where viral binding and detachment from the MIP can be readily identified in the presence of different fluid mechanical shear forces.

To further investigate MIP sensitivity, the viral concentration was reduced to 4 μg/mL (1000-fold decrease) and both native- and imprinted-polymers of the sample arm were continuously perfused over a period of 12 hours. Results in Fig. 6A show no visible virus peak at the native polymer while impedance signals steadily increased over a period of 90 min indicating viral binding events at the MIP. In other words, the contact-less dielectric microsensors normally monitoring both dielectric changes of the buffer solution (to a distance of 2.4 μm in the z-direction) only detected capacitive changes occurring at the 200 nm thick MIP. It is also evident that despite continuous virus perfusion, impedance signals remained constant over a period of 10 hours indicating saturation of MIP binding sites. In a final set of experiments, the ability of the MIP to selectively identify viral contamination was investigated. Here, the sample and reference arms of the microfluidic biochip were loaded with either TMV or HRV2 virus samples and rinsed with buffer solution for over

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**Fig. 5**  (A) Impedance raw data monitored at 252 kHz in the presence of (i) buffer and (ii) 8 μg/mL TMV solution. (B) Background subtracted impedance values in the presence of 35 μm/s and 1750 μm/s (arrow) fluid flows. The arrow shown indicates a 50-fold increase in flow velocity. Virus sample was added after one hour.

**Fig. 6**  (A) Impedance time traces at 203 kHz of 4 μg/mL TMV perfusion in the presence of (i) native and (ii) imprinted polymer. Virus sample was added after 2 hours. (B) Impedance time traces of (1) TMV and (2) HRV2 virus samples in the presence of TMV-sensitive MIP. Virus samples were added after 4 hours.
10 hours. Fig. 6B shows impedance results of 80 μg/mL HRV2 and TMV samples in the presence of TMV-sensitive MIP. Although both contact-less dielectric microsensors detected virus particles present in the buffer solutions, only with TMV-sensitive MIP did a significant signal increase above background levels. These results further demonstrate that, in the presence of microfluidic flow, no unspecific interactions between the HRV2 and the TMV cavities of the MIP took place. Additionally, similar observations have been made with mass-sensitive detectors using the presented MIP layers. For instance, quartz crystal microbalance measurements revealed selectivity factors higher than 10 000 between TMV and HRV for TMV-sensitive MIP. Consequently, recognition can mainly be attributed to the inherent shape differences between the two viruses since the rod-shaped cavities (diameter 18 nm and length 300 nm) in the TMV-sensitive MIP are not able to bind to HRV2 virus particles due to their larger size (approx. 30 nm in diameter). It is important to note that recognition and binding events in the MIP also take place via non-covalent interaction networks which, in the case of viruses, are determined mainly by hydrogen bonding. As a result, despite shape similarities, HRV serotype differentiation has been successfully demonstrated based on small surface chemistry variations. Overall, the low amount of non-specific binding found in the present study is explained by the shape and surface chemistry optimized for TMV–MIP interaction.

In the present study we successfully demonstrate that a two-channel system containing four contact-less dielectric microsensors provides an ideal configuration to continuously detect viral contamination. The combination of microfluidics further provides precise control over fluid dynamic shear forces, thus allowing the investigation of viral binding affinities and dissociation kinetics with the MIP. In addition, observed signal plateaus in the presence of viral samples indicate complete MIP coverage. This means that sensor sensitivity is limited to the size of the MIP and the geometry of the dielectric sensors (μIDC) as well as the intrinsic differences in dielectric constants between the virus and the MIP. Although existing ELISA methods exhibit substantially lower detection limits, the achieved faster response times and reusability of the MIP-Chip allow for continuous monitoring of complex biological samples. Due to its versatility, microchip technology is well suited to detect a broad range of viral contaminations with high sensitivity by selectively adjusting microfluidic conditions, sensor geometries and choice of polymeric material used to fabricate the MIP.

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Conclusion

We have developed an analysis platform capable of continuously detecting viral contaminations that promises to advance existing screening efforts in various human health-related settings. The platform is capable of detecting viral contaminations by employing dielectric measurements of molecularly imprinted polymers (MIPs). The novel combination of four contact-less interdigitated capacitors (μIDCs) with native- and imprinted-polymers integrated into two microfluidic channel networks allows the creation of noise-free detection environments. Complete sensor passivation using approx. 400 nm SiN/ΔSiO2 further eliminates electrode polarization and faradic contributions thus providing drift-free measurement conditions. In the present configuration, mainly capacitive changes caused by viral binding events are responsible for impedance changes. Initially, principal component analysis (PCA) of impedance spectra showed that intrinsic dielectric properties of virus particles can be readily identified using the present sensor geometry. Next, microfluidic experiments in the presence and absence of TMV samples clearly demonstrated that recorded dielectric changes can be associated to viral binding events. The shift in dielectric permittivity is caused by the replacement of buffer with virus particles in the cavities of the MIP and the resulting impedance increase is detected by the contact-less dielectric microsensors. Additionally, frequency analysis using calculated impedance differences prior to and after TMV exposure revealed a distinct maximum at 203 kHz. However, it is not clear if the observed frequency maximum can be linked to the intrinsic dielectric properties of the TMV virus or to interfacial polarization events caused by the surface interactions of the virus with the imprinted polymer charge.

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