



Surface plasmon resonance biosensor for detection of feline calicivirus, a surrogate for norovirus

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ARTICLE INFO

Article history:

Received 12 October 2012

Received in revised form 18 December 2012

Accepted 16 January 2013

Available online 25 January 2013

Keywords:

Intact virus

Norovirus

Surface plasmon resonance

Biosensor

Foodborne

Shellfish

ABSTRACT

The human noroviruses are the most common non-bacterial cause of gastroenteritis and are responsible for as much as 50% of all gastroenteritis outbreaks worldwide. Norovirus (NoV), a single stranded RNA virus, is highly contagious with an infectious dose of less than 100 viral particles. While techniques exist for the identification of NoV, the lack of a reliable cell culture system, NoV genetic variability, and time-consuming sample preparation steps required to isolate the virus (or its genome) prior to molecular based methods has hindered rapid virus detection. To better protect the public from virus-contaminated food and enable better detection in clinical and environmental samples, sensitive and selective methods with simple sample preparation are needed. Surface plasmon resonance (SPR) biosensors represent an emerging detection platform, and this approach has been applied to the rapid detection of foodborne small molecule toxins, protein toxins, and bacteria. This analytical technique, however, has yet to be fully investigated for rapid virus detection, especially for intact viral particles extracted from food matrices. For this study, the culturable, non-human pathogen feline calicivirus (FCV), which has similar morphology and is genetically related to NoV, was chosen as a surrogate virus for designing and evaluating an SPR assay. An antibody-based assay was performed by first immobilizing anti-FCV to an SPR chip surface and then directly measuring virus binding and subsequent secondary antibody binding. The resulting biosensor directly detected intact FCV particles with limits of detection of approximately 10^4 TCID₅₀ FCV/mL from purified cell culture lysates. In addition, intact virus detection in FCV-spiked oyster matrix was possible when using a simple extraction procedure and employing a secondary antibody to FCV for quantitation. The results from these preliminary studies show promise for the development of a rapid assay for detecting intact viruses, such as NoV, using an SPR biosensor. While the current level of sensitivity achieved with this SPR biosensor may be more applicable to virus detection in clinical specimens, broader application and increased sensitivity of this method for foodborne viruses may be achieved when performed in conjunction with efficient virus extraction and concentration methods.

Published by Elsevier B.V.

1. Introduction

Norovirus (NoV) is the most common cause of epidemic gastroenteritis, accounting for at least 50% of all outbreaks worldwide with

approximately 21 million illnesses annually in the United States attributed to this virus (Hall et al., 2011; Scallan et al., 2011). NoV, a non-enveloped, single-stranded RNA virus measuring approx. 30 nm, belongs to the family *Caliciviridae* (Green et al., 2000). This extremely contagious virus has been estimated to be infectious upon exposure to as few as 18 viral particles (Teunis et al., 2008) with transmission generally via person-to-person or foodborne/waterborne contamination. Of NoV illnesses in the U.S., 26% of outbreaks are attributed to foodborne transmission (Scallan et al., 2011) with common products being raw shellfish (Iwamoto et al., 2010; Lees, 2000) and fresh produce (Berger et al., 2010) as well as ready-to-eat foods (e.g., deli meat) handled by an infected individual (Cannon et al., 2006; Terio et al., 2010). In fact, NoV is the leading source of foodborne disease outbreaks in the U.S., with 58% of foodborne illnesses during 2011 attributed to this virus (CDC, 2012; Hall et al., 2011; U.S.FDA) followed to a much lesser extent by hepatitis A virus (HAV) and rotavirus (CDC, 2009, 2010).

Abbreviations: FCV, Feline calicivirus; HBS-N, 0.01 M HEPES pH 7.4, 0.15 M NaCl; HBS-EP+, HBS, 3 mM EDTA, 0.05% (v/v) surfactant P20; LOD, Limit of detection, $\text{AvgRU}_{\text{Blank}} \cdot N = 3 + 3 \times \text{StDev}_{\text{Blank}}$; LOQ, Limit of quantitation, $\text{AvgRU}_{\text{Blank}} \cdot N = 3 + 10 \times \text{StDev}_{\text{Blank}}$; NoV, Norovirus; RU, Resonance Unit; SPR, Surface plasmon resonance; TCID₅₀/mL, 50% tissue culture infective dose per mL.

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The inability to culture human NoV has severely limited progress in characterizing the virus, detecting outbreaks, and evaluating methods for virus inactivation (Cannon et al., 2006; Duizer et al., 2004; Leon et al., 2011). While NoV was first identified using electron microscopy (Kapikian et al., 1972), this time-consuming and insensitive method is rarely used for detection, especially in settings outside of research laboratories. Current detection for NoV generally relies on molecular methods, specifically real-time reverse transcription-polymerase chain reaction (RT-qPCR). This method has the ability to detect very low titers of virus (Kageyama et al., 2003), and the cDNA amplicon can be subsequently sequenced to determine the genotype (Kojima et al., 2002). Unfortunately, virus identification through sample preparation and subsequent detection by RT-PCR methods can be time-consuming (DePaola et al., 2010) and can also suffer from inhibitory substances (factors) that may be present, especially those from food matrices (Butot et al., 2007; Shieh et al., 2000). Enzyme immunoassays (EIAs) can be rapid, less expensive, and less inhibitor prone than PCR. Commercial EIAs for detecting NoV in stool samples are available but often lack the necessary specificity and sensitivity (de Cal et al., 2007). Although EIA has performed well in outbreak cases, a negative EIA should be confirmed by RT-PCR methods due to the potential of the antibodies not having the appropriate reactivity with a specific NoV strain or the viral titer being below the detection threshold (e.g., limit of detection $>10^6$ virus particles per g of feces) (Costantini et al., 2010).

As a non-human pathogen that is easily culturable and has many similar structural features as human NoV, feline calicivirus (FCV) has been used in many studies as a surrogate (Cannon et al., 2006; Duizer et al., 2004). FCV has been used as a process control to assess the extraction and detection efficiency when using a real-time RT-PCR for the detection of RNA viruses (Di Pasquale et al., 2010; Mattison et al., 2009). Similarly, Mattison et al. (2009) suggested that FCV could serve as a quality control for detecting viruses from foods given its relatedness to the enterically infecting viruses, but it has not been directly associated with foods. For these reasons FCV was employed as the surrogate virus for the development of the surface plasmon resonance (SPR) bioassay described here.

An SPR biosensor is a direct, real-time, label-free technique that measures the refractive index change due to biospecific interactions (e.g., analyte binding to its antibody) at or near a thin metal film

assay surface (Homola, 2008; Situ et al., 2010). As shown in Fig. 1, the change in refractive index at the dielectric interface (i.e., gold immunoassay substrate and solution in the flow cell) results in a shift in the SPR band position, which can be tracked with respect to time. These resulting sensorgrams can then be used to determine the amount of analyte binding during an assay by measuring the response (Resonance Unit, RU) after association with the analyte.

In recent years, SPR biosensors have been used to detect many foodborne pathogens including bacteria (Si et al., 2011), nucleic acids from bacteria (Piliarik et al., 2009), protein toxins (Hodnik and Anderluh, 2009; Rasooly, 2001), allergens (Raz et al., 2010), and small molecule toxins (Li et al., 2012; Yakes et al., 2011a, 2011b). However, limited research has been performed using SPR as a diagnostic technique for viruses, with the majority of virus research using SPR sensors focused instead on investigating binding affinity of viral surface proteins (Amano and Cheng, 2005; Caygill et al., 2010). Examples of virus research using SPR detection include the evaluation of antibody interactions with surface-bound viruses (Chenail et al., 2011), determination of antibody affinities for captured influenza A virus (Schofield and Dimmock, 1996), investigation of virus functions (Rich and Myszkowski, 2003), and quantitation of plant viruses (Boltovets et al., 2004). In addition, a quantitative assay for intact influenza virus has been performed with an SPR biosensor; however, this method relied on an inhibition assay and immobilization of virus haemagglutinin (Biacore, 2010). For many analytes, the epitope of antibody binding is not known or the protein is difficult to isolate, and thus an inhibition assay strategy is not universally feasible. Direct SPR detection of the intact form of an insect pathogen, baculovirus, was shown by Baac et al., yet full assay development with quantitation was not pursued in this work due to the limited sensitivity (10^7 pfu/mL) of the system (Baac et al., 2006).

This manuscript presents the development of a quantitative SPR biosensor for intact FCV and shows that by using a secondary antibody, detection of viruses from complex matrices after a simple extraction procedure is possible. While the sensitivity of the assay (on the order of 10^4 50% tissue culture infective dose per mL [TCID₅₀/mL]) is well suited to detect and identify viruses from clinical and environmental samples, application to foodborne virus quantitation from food matrices may be possible when implemented in concert with improved virus extraction and concentration methods.

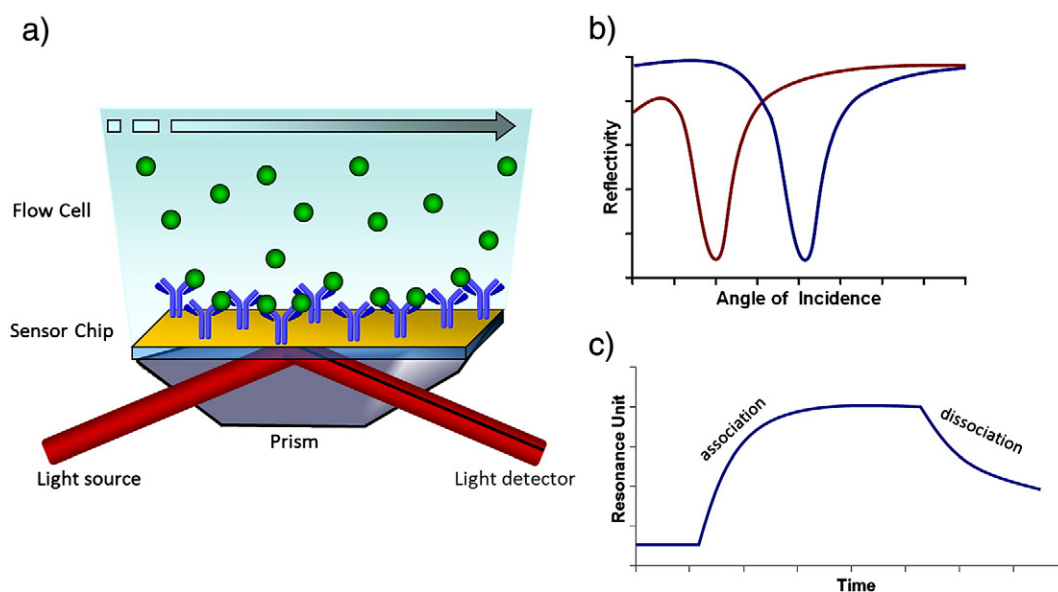


Fig. 1. SPR principle of a direct assay. Binding of the virus (green circles) to the antibody on the sensor chip as shown in (a) shifts the SPR band from the red to blue position as in (b). The change in band position can be evaluated in real-time by plotting the response versus time as shown in (c) with association of the virus to the antibody post injection and dissociation of the virus from the antibody after the injection ends. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Materials

Feline calicivirus (F-9 Strain, VR-782) and its antisera (VR-653 AS/Gt [Ab 653] and VR-956 AS/Gt [Ab 956]) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Millipore water was used for preparation of all aqueous solutions. Oysters were obtained from a local retailer, and the tissue (jar (16 oz.) of whole, shucked, raw oysters) was homogenized using a food processor (KitchenAid Chef's Chopper). Standard materials for the surface plasmon resonance biosensor (including sensor chips, buffers, amine coupling kit, and regeneration solutions) were obtained from GE Healthcare (Piscataway, NJ, USA).

2.2. Virus preparation and titer

FCV stock was propagated in Crandell Reese Feline Kidney (CRFK) cells (ATCC) using Dulbecco's Modified Eagle's Medium (DMEM, Gibco-Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum, penicillin (1000 units/mL), streptomycin (100 µg/mL), L-glutamine, non-essential amino acids and 1.5% sodium bicarbonate. The infectious titer was quantified with a tissue culture infectious dose method (TCID₅₀/mL) as described previously by D'Souza et al. (D'Souza et al., 2006) and Bidawid et al. (Bidawid et al., 2003). The virus was harvested and initially purified as a cell culture lysate with a rapid freeze–thaw and stored in 1 mL aliquots at –80 °C until used. The virus titer was determined using the Reed and Muench method (Reed and Muench, 1938). In order to further purify the FCV, the virus was filtered through a 100 kDa Amicon cutoff centrifugal filtered column (Sigma, St. Louis, MO). Virus standards for SPR analysis were prepared by serially diluting the stock FCV solution in HBS-N buffer with blank samples performed by injecting HBS-N (no virus).

2.3. Antibody preparation for sensor chip fabrication

The stock antibody solutions were prepared by reconstituting the lyophilized antibodies with 1.0 mL water. Since this matrix contains free amines from the original antisera, direct conjugation of the antibodies to the sensor chip may lead to the incorporation of extraneous proteins. To remove these interfering proteins, the antibody solutions were purified using Protein G Agarose with standard procedures (Pierce NAb Protein G Spin Kit, Thermo Fisher Scientific, Rockford, IL, USA). For buffer compatibility with the biosensor fabrication procedure, Zeba Spin Desalting Columns and the Buffer Exchange Procedure (Thermo Fisher Scientific) were used to exchange the elution buffer with a pH 5, 10 mM sodium acetate buffer. Absorbance measurements at 280 nm (Protein A280 IgG method, NanoDrop ND-1000, Wilmington, DE, USA) after purification and buffer exchange yielded concentrations of 3.28 mg/mL and 2.89 mg/mL for antibodies Ab 653 and Ab 956, respectively.

2.4. Biosensor fabrication

A CM3 sensor chip (GE Healthcare) was chosen for the biosensor surface since the shorter carboxymethylated dextran matrix potentially reduces steric effects when working with large analytes (Biacore, 2003). As the dextran monolayer imparts a negative charge to the substrate, antibodies were diluted in 10 mM sodium acetate buffer (pH 5) to allow for antibody pre-concentration on the surface and conjugation without degradation of the antibodies.

After the CM3 chip was normalized following standard Biacore protocols, conjugation was performed using the Amine Immobilization Wizard, HBS-EP+buffer, and associated amine coupling reagents. One flow cell was designed as a reference surface with only

EDC/NHS activation and ethanolamine blocking. This flow cell was then used for bulk refractive index subtraction for the active flow cells during the immunoassays. Active flow cells were conjugated with either Ab 653 (30 µg/mL, approx 8000 RU final conjugation) or Ab 956 (30 µg/mL, approx 4500 RU final conjugation). Prior to use, the sensor chip was conditioned 3× with 10 mM glycine-HCl pH 2.0 (30 s pulses at 30 µL/min), and two start-up cycles with HBS-N were performed.

2.5. Preparation of FCV in oyster matrix

To prepare the oyster matrix, a modified extraction procedure from that previously employed in SPR detection was used (Campbell et al., 2010). One gram of oyster homogenate was extracted with 4 mL of HBS-N, vortexed for 30 s, placed on a roller mixer for 30 min, and centrifuged at 3000 g at room temperature for 10 min. The supernatant was decanted and spiked with the appropriate amount of FCV to be used for the stock calibration solution (3.4×10^6 TCID₅₀/mL) which was then used in 5-fold, serial dilutions in oyster matrix to prepare the other standards. Oyster matrix with no FCV added served as a blank. To assess the recovery of the virus from the oyster matrix, a sample was prepared by spiking one gram of oyster homogenate with 50 µL of 1.7×10^7 TCID₅₀ FCV/mL prior to extraction. In addition, oyster homogenate was spiked with 50 µL of HBS-N prior to extraction to serve as an uninoculated sample (0 TCID₅₀ FCV/mL).

2.6. Immunoassay

Initial experiments evaluated three FCV treatments to determine which form of preparation yielded the best SPR response: firstly, FCV was used as cell culture lysate without any further cleanup; secondly, the cell culture lysate was filtered through a 100 kDa cutoff membrane; and thirdly, the lysate was first filtered as before and further concentrated by ultracentrifugation. These studies showed that filtered FCV yielded the highest sensor response (data not shown) and was therefore used for the rest of the studies. Additionally, buffer free of surfactants (HBS-N) was used for all assays.

The immunoassay flow rate, FCV contact time, operating temperature, stabilization time, and regeneration conditions were also investigated prior to performing full assays. Specifically, flow rate studies were performed at 5, 15, and 75 µL/min with injections of 3.4×10^5 TCID₅₀ FCV/mL. Data from this investigation indicated that higher binding (approx. twice the response) occurred at slower flow rates, and as such 5 µL/min was used as the FCV injection flow rate. In addition, full assays were performed at both 25 °C and 35 °C, but as the higher temperature offered no improvement in response, subsequent assays were performed at 25 °C.

Regeneration of the sensor surface removed bound virus and secondary antibody, creating a clean primary antibody surface for the next analysis cycle. To determine the solution that allowed for complete removal of the virus/secondary antibody while not degrading the biosensor activity, experiments were performed using multiple regeneration solutions. Sodium hydroxide solutions were too harsh on the substrate and led to removal or degradation of the primary antibody. Glycine-HCl solutions at pH ≥ 2.5 did not fully remove the virus/secondary antibody complex. The most complete removal of virus/secondary antibody, while maintaining a stable baseline, was obtained with 10 mM glycine-HCl, pH 2.0, and this was used for all further assays.

Assays were performed with virus standards injected over the chip for 420 s at 5 µL/min. Following a 30 s stabilization period, secondary antibody (1:125 dilution of antiserum in HBS-N, filtered through a Costar 0.22 µm syringe filter) was injected for 90 s at 10 µL/min. Regeneration was performed with a 120 s (30 µL/min) injection of 10 mM glycine-HCl, pH 2.0. The sample compartment was set at 10 °C with an assay temperature of 25 °C.

2.7. Instrumentation and data processing

All biosensor fabrication and immunoassays were performed on a Biacore T100 (GE Healthcare) SPR instrument, which was operated via the Biacore T100 Controller Software v. 2.0. Biacore T100 Evaluation Software and GraphPad Prism (v. 5.02, La Jolla, CA, USA) were used for data processing and calibration curve generation. Curves were fit using a one-site, specific binding with Hill Slope model.

3. Results and discussion

For rapid sample evaluation, immunoassays incorporating minimal sample preparation are necessary. As such, this SPR biosensor was designed to detect intact viral pathogens in a direct immunoassay format with additional binding analysis via a secondary antibody injection. As shown in Fig. 2a, the virus sample was injected over the sensor substrate that had anti-FCV coupled to the surface. When the virus bound to the surface, the corresponding signal (response in RU) increased (Fig. 2b). As expected in this direct assay, higher concentrations of FCV led to more binding and a corresponding increase in RU. Taking advantage of the multiple FCV epitopes that the antibodies can bind to, a secondary antibody was injected over the captured FCV on the sensor surface, and an increase in response was observed (Fig. 2b), with the amount of secondary antibody binding being directly proportional to the original FCV solution concentration. Finally, the sensor surface was regenerated back to the original primary antibody substrate by breaking the virus and primary antibody interactions, thereby allowing multiple analyses per sensor chip. The sensor chip showed excellent binding stability and assay repeatability over time. For example, when a 16 cycle (virus injection, secondary antibody injection, and regeneration per cycle) concentration series was run twice, there was on average a 2.8% change in signal for the duplicate measurements of FCV binding on the Ab 653 flow cell and a 1.8% signal change for FCV binding on the Ab 956 flow cell.

In order to evaluate the sensorgrams, the reference flow cell response was subtracted to account for bulk refractive index variations. Report points were set for each cycle at 0 s (Baseline 1), 30 s after the injection stops (FCV binding), 10 s prior to secondary Ab injection (Baseline 2), and 30 s after the secondary Ab injection stops (Ab binding) with each having a 5 s window (Fig. 2b). To generate FCV

calibration curves (Fig. 3a), the response was determined by subtracting the Baseline 1 response from the FCV binding response, and the signals were normalized to the response of the highest FCV concentration (8.5×10^6 TCID₅₀/mL). For the determination of the secondary antibody calibration curves (Fig. 3b and 3c), normalization was performed similarly. The exception was that the non-zero initial baseline (at Baseline 2) was taken into account by subtracting the response from a blank injection from that of each measurement. These normalized values were then plotted versus the FCV sample concentration in TCID₅₀/mL (log scale) with the best fit to the data via a one-site, specific-binding model with Hill Slope (GraphPad Prism).

For direct binding analysis, there was little difference between the calibration curves for the two, high-density antibody surfaces designated Ab 653 and Ab 956 in Fig. 3a. The limit of detection (LOD, $\text{AvgRU}_{\text{Blank}} + 3 \times \text{StDev}_{\text{Blank}}$) and limit of quantitation (LOQ, $\text{AvgRU}_{\text{Blank}} + 10 \times \text{StDev}_{\text{Blank}}$) were calculated for each curve. The LOD and LOQ for Ab 653 surface were 2.6×10^4 TCID₅₀/mL and 9.6×10^4 TCID₅₀/mL, respectively. For the Ab 956 surface the LOD was 2.3×10^3 TCID₅₀/mL with an LOQ of 9.7×10^3 TCID₅₀/mL. The improved LOD and LOQ for the Ab 956 surface were due to lower standard deviation (0.5 RU versus 1.0 RU) from duplicate measurements on this surface. In addition, the same antibodies used for surface modification were used as the secondary antibodies in the sandwich immunoassay, with the associated calibration curves in Fig. 3b and 3c. In all cases, the detection limits are on the order of 10^4 TCID₅₀/mL. For the secondary antibodies, the higher standard deviation, especially seen at low FCV concentrations, may hinder quantitation, and actual detection capability may be less sensitive than that calculated purely from blank measurements. We attribute this increased error in these low concentration measurements to the complicated kinetics of the interaction, as can be seen in Fig. 2b where dissociation of the FCV from the surface-bound antibody is occurring simultaneously as the association phase of the secondary antibody is taking place. When performing assays in matrices that have been well matched to the running buffer, thus decreasing challenges with bulk refractive index changes and potential non-specific binding, analysis would likely be more reliable using the FCV direct-binding portion of the sensorgram.

Many samples, however, are not amenable to complete buffer matching, and to further investigate assay performance, a complex

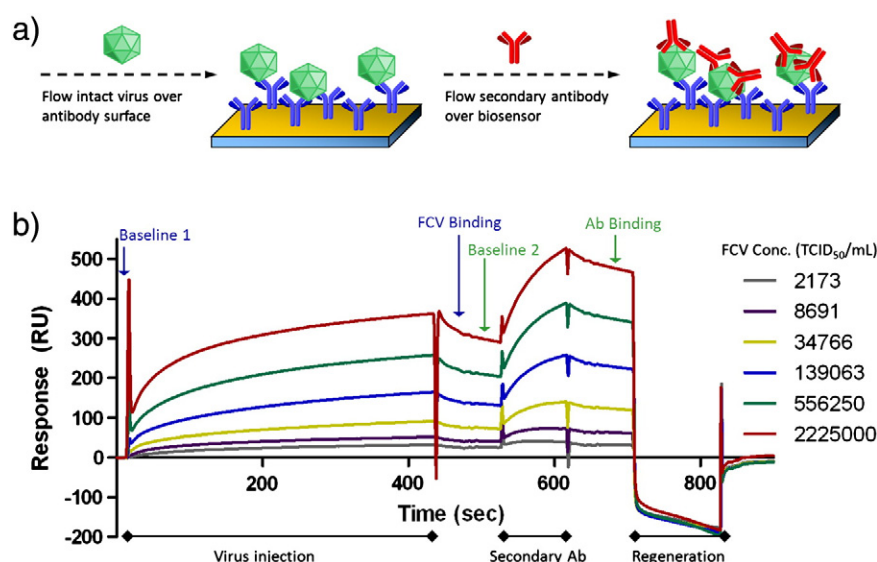
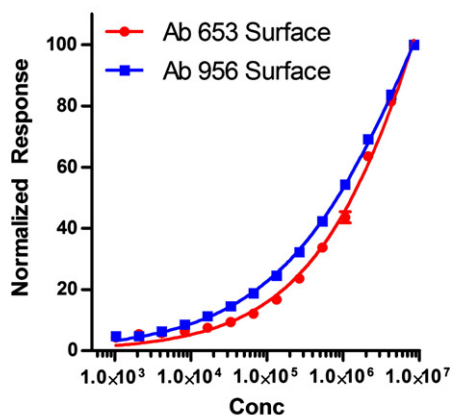
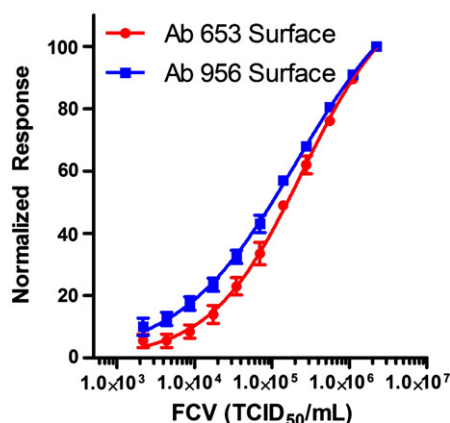


Fig. 2. (a) The FCV assay was performed by injecting intact virus over the antibody sensor chip followed by the injection of a secondary FCV antibody. (b) The sensorgram (reference flow cell subtracted) for a series of individual FCV and secondary Ab injections. With higher concentrations of virus, more binding to the sensor surface and a greater response (RU) for both the virus and secondary Ab binding were observed. After the assay was complete, the sensor chip was regenerated using 10 mM glycine-HCl (pH 2.0) to remove the virus and secondary Ab from the chip surface without degrading the primary antibody on the sensor chip.

a) Direct Assay



b) Secondary Ab653



c) Secondary Ab956

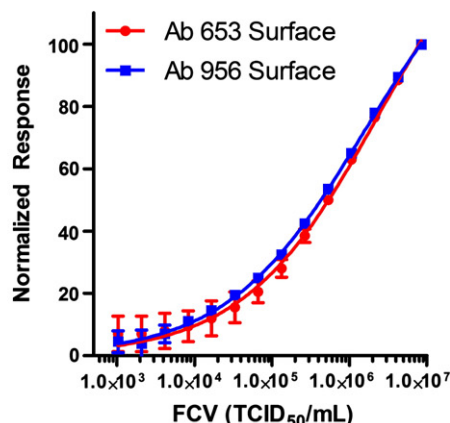


Fig. 3. Calibration curves for (a) direct virus detection on Ab 653 and Ab 956 surfaces and secondary antibody injections (Ab 653 in (b) and Ab 956 in (c)). Each data point is from the average of two technical replicates with standard deviation shown in the error bar. The best-fit line is from a one-site, specific-binding with Hill Slope model (GraphPad Prism).

matrix was evaluated. Viral illness outbreaks have been associated with the consumption of contaminated shellfish, specifically raw or undercooked oysters (Alfano-Sobsey et al., 2012), and one challenge to detection can be from inefficient particle recovery from the tissue or interference from the matrix during pathogen detection. Recently, Schultz et al. compared four oyster sample treatment protocols commonly used for NoV RNA extraction followed by RT-PCR detection, and evaluated the methods based on recoveries and quantitation

(Schultz et al., 2007). Of those evaluated, the overall superior method involved almost 1 day of preparation, using tissue disruption and RNA extraction with a commercial RNA kit and centrifugation. This buffer method showed good recovery and limited inhibitory factors for PCR with the added benefit of avoiding high amounts of organic reagents. In general, SPR analysis performs best when using similar extraction (i.e., homogenization, buffer extraction, and centrifugation to remove solids). As such, a proof-of-concept matrix study using a simple extraction method modified from Campbell et al. was investigated (Campbell et al., 2010). While the original Campbell et al. extraction procedure was developed for toxin analysis, this simple extraction has been shown to have limited matrix effects when performing SPR assays. When applying this simple extraction procedure to viruses, a neutral pH buffer (SPR running buffer HBS-N) was substituted for the pH 5 sodium acetate buffer, which was intended for stabilizing seafood toxins.

While FCV is not expected to be a natural contaminant of seafood, FCV has been used extensively as a surrogate for human NoV (Duijzer et al., 2004; Mattison et al., 2007). Accordingly, FCV was spiked into the oyster matrix to evaluate the potential for virus detection from a seafood matrix. The sensorgram and associated calibration curve for virus detection from oyster matrix are shown in Fig. 4. When evaluating solely the virus injection portion of the sensorgram (–500 to 0 s, Fig. 4a) no trend in FCV binding was observed due to the large nonspecific binding of the oyster matrix and bulk refractive index changes. Such matrix interference may be eliminated simply by diluting the oyster supernatant (e.g., 1 in 20 in buffer) (Campbell et al., 2010), but this would also limit the sensitivity of the assay. To eliminate the need for matrix dilution, secondary antibody binding was also investigated. The secondary antibody binding, shown in Fig. 4a from 0 to 150 s, showed a clear concentration trend with respect to the

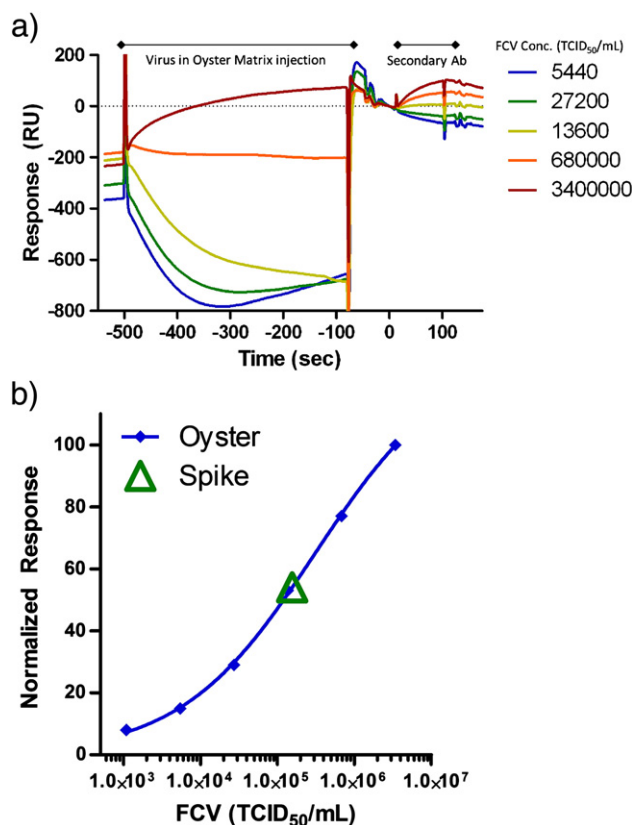


Fig. 4. (a) Sensorgrams of virus-spiked oyster matrix injections, followed by secondary antibody (Ab956) performed on Ab 956 surface and (b) associated calibration curve and spiked-sample position on the curve from the signal of the secondary antibody binding (N = 1).

original FCV solution concentration. In addition blank oyster samples (0 TCID₅₀/mL) showed no binding from the secondary antibody over secondary antibody binding from a buffer blank. From the binding response of the secondary antibody, a calibration curve (Fig. 4b) was obtained and then used to calculate the concentration of an unknown sample. In this case, the experimentally determined concentration for the FCV-spiked oyster homogenate (1.56×10^5 TCID₅₀/mL) matched well with the 1.67×10^5 TCID₅₀/mL spiked into and extracted from the virus matrix. Furthermore, the uninoculated oyster extract (0 TCID₅₀/mL) showed no signal over the blank. These promising results indicate that this simple extraction procedure in concert with secondary antibody binding may allow for accurate, quantitative detection of viruses using SPR without extensive sample preparation techniques.

4. Conclusions

In this study, a quantitative technique for the detection of the NoV surrogate FCV was developed using an SPR biosensor. To our knowledge, this is the first time that an SPR assay has been designed to detect viral pathogens from seafood. This rapid method has a complete assay cycle (i.e., FCV-direct detection, secondary antibody injection, and surface regeneration) of less than 15 min, has LODs on the order of 10^4 TCID₅₀/mL, and shows promise for detecting intact viruses from shellfish following a simple extraction procedure. While the detection limits for whole virus detection obtained in our study were comparable to or better than other immunoassay techniques (e.g., approx. 10^6 to 10^7 virus particles g⁻¹ of fecal sample (Costantini et al., 2010)), the sensitivity is not yet amenable to use for direct virus detection from food at levels that could cause illness (e.g., approx. 100 viruses). However, viral levels in clinical and environmental samples (e.g., contaminated water) are typically much higher than those in food (Butot et al., 2007), and the SPR assay could potentially be applied for rapid NoV detection in these sample types. For assay use in these instances, viral binding studies (e.g., capsid integrity and infectivity assays) would be necessary. It is also important to note that SPR biosensor assays rely on antibodies, and further development of specific antibodies, or alternative analytical recognition elements and affinity ligands (e.g., histoblood group antigens for NoV), would also be necessary to overcome the lack of strain identification and cross reactivity challenges seen for human NoV. While this current SPR biosensor utilizes similar antibodies to EIAs, SPR technology offers the advantages of being a label-free, near real-time assay that can provide rapid and potentially multiplexed-viral pathogen analysis. Additional research in the development of faster and more sensitive upstream processing approaches to isolate viruses from foods, including improving extraction techniques, concentrating viral particles (e.g., via ultracentrifugation (Albinana-Gimenez et al., 2009) or hollow-fiber filters (Smith and Hill, 2009)), or enhancing immunoassay signal may further improve the LOD achieved for the SPR biosensor developed herein.

Acknowledgment

Thank you to Samantha M. Prezioso for her contributions to virus biosensor work at CFSAN.

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