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Development of a label-free and innovative approach based on surface plasmon resonance biosensor for on-site detection of infectious bursal disease virus (IBDV)

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ABSTRACT

An innovative, specific and label-free detection approach based on optical surface plasmon resonance (SPR) was developed and employed in the development of a rapid and quantitative bioanalyzer for detecting infectious bursal disease virus (IBDV) in the field. A unique bioanalyzer based on this approach was established which consists of a micro-flow cell, a temperature regulator, an integrated biosensor, an optical platform, an electronic control unit incorporated into a photoelectric conversion device, and a universal serial bus (USB) interface circuit board. The procedure for detecting IBDV was systematically described, and experimentally validated. The self-assembly technology was used to make the IBDmAb adhere to the surface of the sensor chip by a bifunctional cross-linker. By this approach there exhibited a linear relationship between the IBDV concentrations and the corresponding responses in the range of dilution factors from 100 to 1600 with R^2 0.97982. We were able to detect 400-fold diluted IBDV using this biochip repeatedly with a calculated relative standard deviation (RSD) of 3.6%. We also showed that the detection limit of the SPR biosensor biochip was around 1/18 of the detection limit of the IBDV diagnostic strip. Satisfactory recoveries were obtained from the recovery test. The approach presented here was shown to have great potential to be used in the IBDV epidemic regions and hence help to promote the effective implementation of sound control strategies against IBDV.

1. Introduction

Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious disease of young chickens caused by infectious bursal disease virus (IBDV), characterized by immunosuppression and mortality generally at 3–6 weeks of age (Muller et al., 2003). The immunosuppression leads to an increased susceptibility to other pathogens and reduces the growth rate of surviving animals (Khatri and Sharma, 2009). Adequate control of IBDV is highly contingent on the availability of rapid and efficient diagnostic procedures and approaches for identifying IBDV. Over the past years, many attempts have been made to detect IBDV. An enzyme-linked immunosorbent assay using the expressed protein of IBDV as the coating antigen for detecting antibodies binding IBDV has been developed (Saravanan et al., 2004). A rocket immunoelectrophoresis test has been used for the qualitative detection and quantitative estimation of IBDV specific antigen in experimentally infected chickens (Dhinakar Raj et al., 2000; Palarasah et al., 2010). A real-time polymerase chain reaction (PCR) method has also been developed for the detection of IBDV (Mittal et al., 2005; Ghorashi et al., 2011).

Despite the aforementioned techniques, there is still a lack of rapid and quantitative tools for measuring IBDV. Biosensors are analytical devices that integrate biological sensing elements for detecting an analyte through the combination of a series of physicochemical transductions such as optical, electrochemical, or piezoelectric detection (Yakes and Mossoba, 2010). Over the last 20 years, immunosensors using surface plasmon resonance (SPR) have been developed for measuring antigens binding to antibody molecules immobilized on the SPR biosensor surface with high specificity and sensitivity (Sakai et al., 1998; Liu and Wilson, 2010). However, there has been so far no published study on the detection of IBDV using the SPR immunosensors. In a previous study, we reported the use of a portable home-made SPR biosensor system in a low-cost bioanalyzer measuring molecular interactions between virus and antibody (Hu et al., 2009). In this study, we present an innovative label-free detection system to use SPR biosensor in a novel platform specifically for on-site detection of IBDV. The results obtained indicate that this method has great potential to be used in IBDV detection.

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2. Experiment

2.1. Materials

In this study, the three-channel Spreeta modules (TSPR1k23) manufactured with a gold slide bonded to the sensor modules were obtained from Nomadics, Inc. (Stillwater, USA). The microelectric valve actuator was acquired from VICI Valco Instruments Co. Inc. (Houston, USA). Mercaptopropionic acid (MPA, 0.04 M), amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide–HCL (EDC, 0.4 M), N-hydroxysuccinimide (NHS, 0.1 M), and ethanolamine–HCL (1 M, pH 8.5) were purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). The IBDV (TCID50 = 105.57%, 50% tissue culture infective dose) and the anti-IBDV monoclonal antibody (IBDmAb) were provided by Henan Key Laboratory of Animal Immunology (Zhengzhou, China).

2.2. Construction of experimental platform

The analyte assay was carried out on the SPR experimental platform. The dimension of the setup is 30 mm × 20 mm × 16 mm and 3.4 kg in weight (Supplementary Fig. S1). The core component of the SPR experimental platform is the integrated biosensor TSPR1k23 which is built based on the diverging fan-shaped beam configuration. No angle-controlled scanning device is adopted in the SPR experimental platform. The position of the minimum light intensity moves over the biosensor chip while the biomolecular interactions proceed. A linear charge-coupled device (CCD) was used to monitor the light intensity reflected from the thin Au film. The signal responses with respect to different light intensities vary with pixel position. The schematic illustration of this experimental platform is shown in Fig. 1.

The IBDV analytical and experimental platform was constructed through the following steps: (a) fixing a Spreeta biosensor (TSPR1k23) onto the clamp; (b) functionalizing the biosensor in each channel by attaching at least one molecule to the surface of the biosensor; (c) implementing a very precise control of the sample flow rate actuated by the micro-pump; (d) passing samples through the microelectric actuator to the biosensor; (e) displaying the RU–t curve compiled in Delphi 7.0 on the computer screen; (f) setting up a temperature control module for measuring the chamber temperature and keeping it constant at 25 °C in real time; and (g) laying an optical biosensor platform for clamping the TSPR1k23 biosensor, micro-flow cell and electrical control board. The SPR response signal analysis, curve plotting and biomolecular dynamic fitting are performed by a computer automatically. A new biosensor chip was fabricated in the laboratory when the Au film was scratched or damaged. The chip was composed of a BK7 glass substrate and a 50 nm Au film which was deposited on the BK7 glass substrate by electron beam evaporation.

The fluidic schematic shown in the left part of Fig. 1 illustrates how the microelectric actuator was switched to perform washing steps, collecting sample solution and finishing detection. In the indirect inhibition immunoassay, the fluidic system can channel the amplifying reagents to flow through over the biosensor surface or to purge the sample loop with a buffer or deionized water in order to wash the biosensor surfaces and prevent cross-contamination (Chinowsky et al., 2007). The sequence of basic steps is first to collect a sample, then to flow the sample over the biosensor surface, and then to determine the rate of binding of IBDV to IBDmAb immobilized on the biosensor surface, and finally to calculate the concentrations of IBDV from the standard curve already established in the computer memory. The schematic diagram of this temperature control module and the IBDV detection configuration are shown in the supplementary data (Figs.S2 and S3). The temperature regulator consists of two temperature control modules (TEC-12705, Sunner Hitec Co., Ltd., Shanghai, China) and one temperature control module with a proportional–integral–derivative (PID) controller was used to keep the temperature at 25 ± 0.1 °C. To avoid environmental influence on the experiments conducted in the chamber, an aluminum housing was insulated in a two-layer board for better temperature control, which reduced the electrical supply power consumption from the temperature control module (Naimushin et al., 2003).

As shown in Supplementary Fig. S3, the IBDV detection configuration consists of a fixed clamp for holding biosensor TSPR1k23, a fixed part for holding micro-flow cell, a draw rod and a bearing base. The micro-flow cell was arranged on top of the Au film surface of the biosensor and can be lifted up and down to an arbitrary position along with the fixed part by adjusting the draw rod. When the desired position was reached, the micro-flow cell can be latched by the fixed knob.

2.3. Biosensor membrane preparation

As a base layer, the self-assembled monolayer allows the attachment of various elements to the SPR biosensor surface (Hegnerova and Homola, 2010). In our experiments, a self-assembled monolayer membrane of mercaptopropionic acid (0.04 M, pH 5.0) was firstly formed on the surface of Au film through gold–sulfur interaction to capture the IBDmAb to the biosensor's surface. IBDmAb was then immobilized on the biosensor chip through its interaction with the MPA at 25 °C. The designated micro-flow cell was activated with a mixture of EDC and NHS (1:1, v/v) for about 1 h. The antibody solution (diluted by 100-fold with 10 mM sodium acetate, pH 4.5) was then injected to the micro-flow cell and its adsorption was monitored until the surface was saturated, and no changes of signal were observed with time. The resonant pixel position changes were reported as response units (RUs). RUs were computed based on the following formula (Chinowsky et al., 2003).

$$\mathrm{RI}_x = C_0 - C_1 \times \mathrm{PP} \tag{1}$$

where RI_x is the refractive index of an unknown sample, $C_0 = 1.36476912$, $C_1 = 4.37627713 \times 10^4$ are the constants determined by the SPR configuration, and PP is the pixel position number obtained from the linear CCD sensor.

$$\mathrm{RU} = (1.334 - \mathrm{RI}_x) \times 30,000 \tag{2}$$

where 1.334 was the refractive index of deionized water and 30,000 is a pre-determined factor for increasing the sensitivity of the calculated responses.

Finally, unreacted ester functionalities were deactivated with ethanolamine (1 M, pH 8.5) for about half an hour. Following immobilization, the biochip was stored at 4 °C in a sealed container. The schematic illustration of the stepwise immobilization of IBDmAb is shown in the supplementary data (Fig. S4).

All measurements were carried out under 25 ± 0.1 °C. Supplementary Fig. S4 shows the SPR measurements of the binding of molecules during the pretreatment of the biosensor surface. The RUs were displayed on the computer screen while the MPA (0.04 M) was injected through the micro-flow cell at 0.5 rpm. In order to modify the biosensor surface, the carboxyl groups of a thiol monolayer were activated with freshly mixed 0.4 M EDC/0.1 M NHS (1:1) in water, for a single or three successive periods of 5 min. The response signals became fluctuating due to bubbles generated during the activation process. After the activation and a brief wash with deionized water, an IBDmAb solution was injected until the response signals reached a steady state, and then a 1 M ethanolamine–HCL buffer (pH 4.5) was applied to the biosensor surface to seal redundant binding sites to

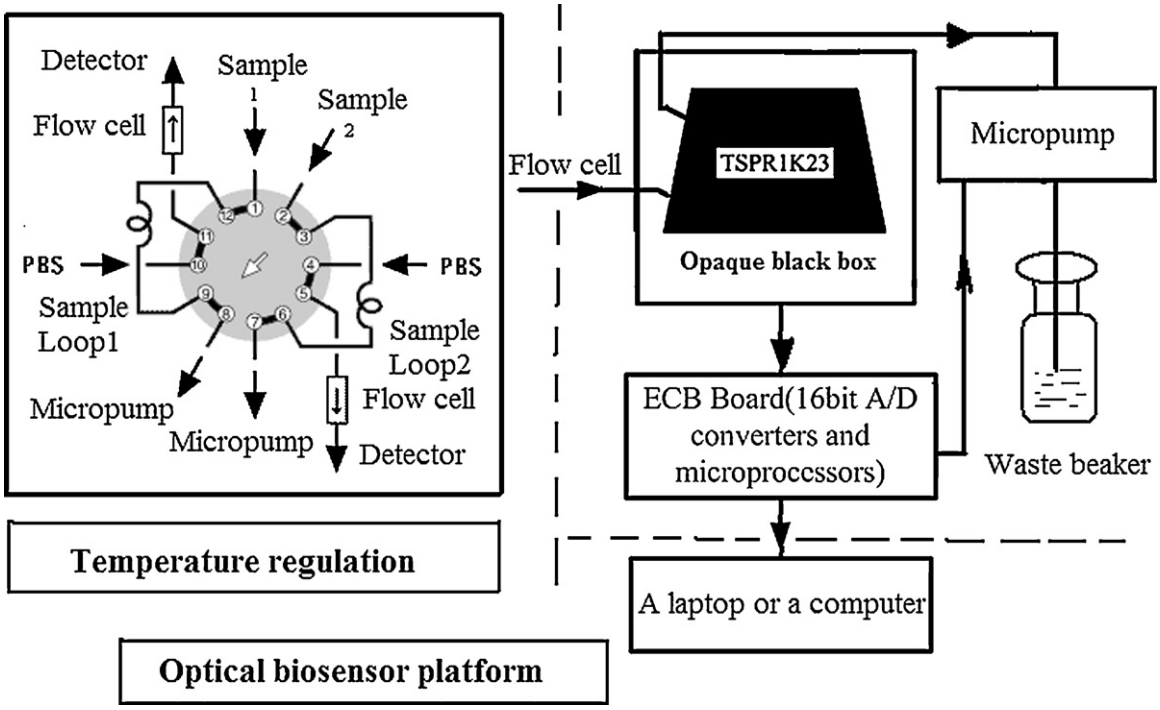

Fig. 1. Schematic configuration of the SPR experiment platform using the integrated module TSPR1k23 for monitoring the responses of IBDV.

improve the efficiency of immobilization for a whole 15 min, or three successive 5 min.

3. Results and discussion

3.1. Detection and analysis of the infected BDV sample

Following the basic principle of IBDmAb–IBDV interaction kinetics, the corresponding binding curves for serial dilutions of IBDV with immobilized IBDmAb were obtained by this approach, with each RU–*t* curve indicating the intensity of response to a specific concentration of IBDV until the plateau of IBDmAb–IBDV immunoreaction on the biosensor surface is reached. Initially the phosphate-buffered saline (PBS) flowed over the biosensor surface. The background response signals were produced by injecting the PBS buffer over the biosensor surface. When the sample containing the IBDV was injected, the upward slope of the curve indicates the association of the IBDV with the immobilized IBDmAb. The ΔRU was the value obtained by subtracting the background response signals from the sample response signals or standard response signals. Fig. 2 shows the sensorgram of five typical binding curves, denoted by 1/100, 1/200, 1/400, 1/800, and 1/1600, showing the kinetic behavior of different concentrations of IBDmAb–IBDV. The TCID50 values calculated using the Reed–Muench formula (Schmidt and Emmons, 1989) were 3715, 1858, 928, 464, and 232, corresponding to 100, 200, 400, 800, and 1600-fold dilutions, respectively.

From Fig. 2, the descent of response units (ΔRU, actual binding responses) obviously decreased with increasing diluted factors, or with decreasing concentration of IBDV. The differences between any two adjacent curves in Fig. 2 were apparent, implying that this approach has a high sensitivity. The superiority of this approach lies in its continuous measurement, where several samples could be detected by the same biosensor chip and the time required from the interaction to the distinction between various concentrations was less than 20 min.

Fig. 3 showed the response unit differences (ΔRU) after 350 s for different dilution factors (or concentrations). A second order polynomial regression yielded a high R^2 value of 0.97982, where the operating range of dilution factors was optimized to 100–1600. The corresponding dilution factor sensitivity was 63.607 (RU/dilution factor). To test the repeatability of the measurements, 400-fold diluted IBDV was measured 5 times and the results were shown in Supplementary Fig. S5. The baseline represents a continuous flow of PBS buffer. An increase in response signals (association) was observed when the IBDV binds to the IBDmAb membrane immobilized on the surface of the biosensor chip. At the end of the

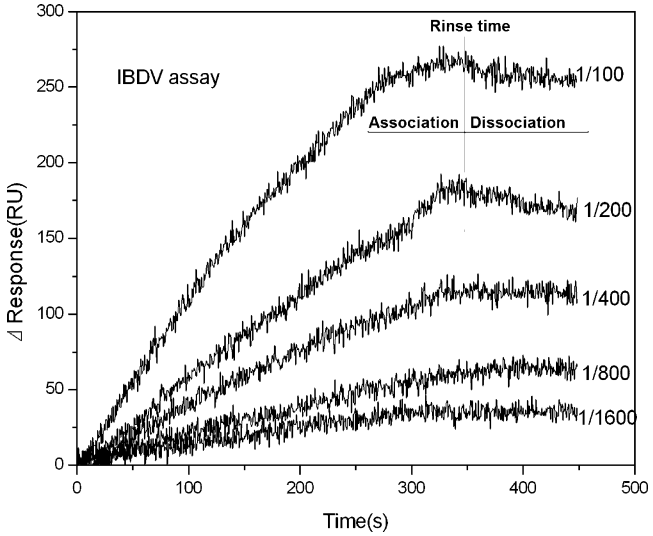

Fig. 2. Surface plasmon resonance sensorgrams generated from detection of IBDV. The 100-fold, 200-fold, 400-fold, 800-fold and 1600-fold dilution of IBDV using PBS buffer were flowed over the immobilized IBDmAb. The zero of "time" axis was set at the point of time at which the association between the two biomolecules starts to occur. The 350 s was the time when an equilibrium state plateau was reached.

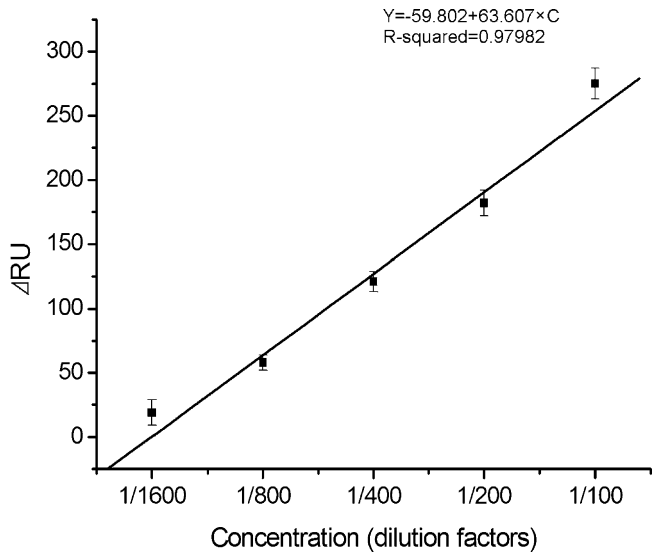

Fig. 3. Measurement results of the response differences versus different dilution factors (or concentrations).

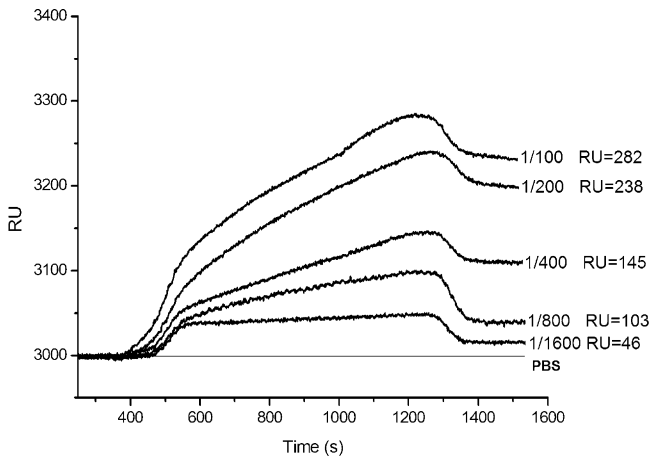

Fig. 4. Representative sensorgrams of IBDV detection from a chicken tissue sample. Curves for 100- and 1600-fold dilutions are presented as an average of 5 independent measurements. A baseline was created by injecting PBS buffer over the surface of the biosensor chip for 6 min.

injection the sample was replaced with a continuous flow of the PBS buffer and the decrease in response signals (dissociation) reflects the dissociation of the IBDV from the IBDmAb. A regeneration solution is injected to dissociate the remaining IBDV. The ΔRU of these 5 times assay were 150, 141, 148, 141, and 137, respectively. Statistical analysis indicated that the relative standard deviation (RSD) was 3. 6%.

3.2. Comparison analysis with the diagnostic strip

In order to compare the sensitivity and specificity of this biosensor system and the commercial diagnostic strip for on-site detection of IBDV, an IBDV sample was diluted to 100-, 200-, 400-, 800-, 1600-, and 3200-fold with PBS buffer. Under the same condition, all dilutions were measured by the biosensor system and IBDV diagnostic strips (Henan Key Laboratory of Animal Immunology, Zhengzhou, China), respectively. It was found that the biosensor system detected sample dilutions to as low as 1600-fold (see Table 1). In contrast, IBDV diagnostic strips failed to detect IBDV beyond the 100-fold dilution. The lower limits of detection (3 S/N) for the biosensor system and the diagnostic strip were around 2.5 ng/ml and 46 ng/ml, respectively.

3.3. Analysis of a chicken tissue sample

It is preferable that chicken tissue samples (IBDV infected) were processed immediately after removal from the freezer, because thawing will produce a rubbery nugget that evades slicing or dicing. IBDV infected tissue solution was prepared as follows: (1) place a weighed tissue in a mortar and add an appropriate amount of PBS to achieve a mixed sample at the ratio of 1:10; (2) cut the chicken tissue into fine pieces to ensure the complete extraction of IBDV; (3) add an appropriate amount of SiO_2 powder and grind; (4) transfer the tissue homogenate into the centrifugal tube immediately and centrifuge for 5 min at 3000 rpm; (5) transfer the supernatant into a new centrifugal tube for later use. The RU value of the sample was detected to be 2334 using the biosensor system. As RU is a measure of the relative concentration of viruses, this high RU value indicates a relatively high IBDV concentration in the supernatant. The supernatant was diluted by 100-, 200-, 400-, 800-, and 1600-fold with PBS before analysis.

The ΔRUs are 282, 238, 145, 103, and 46 corresponding to 100-, 200-, 400-, 800-, and 1600-fold of dilution, respectively (see Fig. 4). It was clear that the concentration of IBDV could be measured by the biosensor system (Fig. 4).

3.4. Recovery test

A recovery test is a bioassay for determining the efficiency of a biosensor system in detecting a specific analyte. The recovery of the analyte is the difference between the signal responses prior to and after a known dose of the analyte is added to a biological matrix. This value is then compared against the standard signal response, which is obtained from a standard buffering medium to which an identical dose of the analyte is added. The recovery rate, a measure of the detecting efficiency, is then calculated from the following formula.

$$C = \frac{(RU_2 - RU_1)}{RU_3} \times 100\% \quad (3)$$

where C is the recovery rate, RU_1 is the response of the sample prior to the addition of the analyte, RU_2 is the response after the addition of the analyte, and RU_3 is the standard response.

RU is derived from the refractive index of the immediate vicinity of the biosensor surface. The refractive index of a prepared chicken tissue sample is dependent on the density and other properties of the prepared sample, hence, the value of RU may vary with different sample preparations and different chicken tissues. In order to determine the effectiveness of our biosensor system in detecting IBDV infection, we tried to eliminate the variation in response between samples by comparing the biosensor response of a sample prior to IBDV infection with the response of the same sample after infection. Each IBDV-free sample was first detected three times, and then IBDV of known concentration was added to that sample and tested again three times.

The result showed that biosensor responses of each sample prior to infection and after infection of IBDV are clearly different from each other (Table 2), as can be discerned from the small values of RSD of the three runs of the same sample. In the first three samples of chicken tissue, a 500-fold dilution of the raw IBDV was added; the means of detected responses (ΔRU) for the three samples were 133, 127, and 114, respectively, as compared with the standard response (ΔRU = 114) for the same IBDV dilution. In the second three samples, a 600-fold dilution of the raw IBDV was added; the means of detected responses (ΔRU) for the three samples were 96,

Table 1
Comparison of the sensitivity and specificity between the SPR biosensor system and IBDV diagnostic strip.

Diluted factors	Original IBDV sample solution	10	20	40	80	100	200	400	800	1600	3200
SPR biosensor system	+	+	+	+	+	+	+	+	+	+	–
Diagnostic strip	+	+	+	+	+	+	–	–	–	–	–

+: indicating positive sign; –: indicating negative sign.

Table 2
Results of the chicken tissue sample recovery test.

Sample	Responses of the IBDV free sample[a]		Responses after Adding IBDV		Detected responses	Standard responses	Recovery rate
	Mean of three tests	RSD	Mean of three tests	RSD			
1	291	4.56%	424	4.41%	133	114	116.66%
2	297	5.73%	424	2.88%	127	114	110.40%
3	333	8.40%	447	3.42%	114	114	100%
4	233	1.97%	329	1.35%	96	93	103.22%
5	240	3.12%	339	2.48%	99	93	106.45%
6	259	3.57%	364	2.31%	105	93	112.90%

[a] An IBDV-free sample was first detected three times, and then an IBDV sample of known concentration was added to that sample and tested three times again. The mean and relative standard deviation (RSD) of each three runs was calculated. The detected responses were the difference between response from the IBDV-free chicken tissue sample and that from the sample with known concentration of IBDV. The standard responses are responses from standard solution of the known concentration IBDV.

99, and 105, respectively, as compared with the standard response (ΔRU = 93) for the same IBDV dilution. These data showed that the recovery rate is very satisfactory despite the variation in the background (IBDV free) responses among samples. The small value of the RSD of each three runs indicates that responses from repeating detections of the same sample were satisfactorily stable. As we did not pay much attention on standardizing the preparation of the test samples, there seems to be a considerable variation in the background RUs among different samples. We expect that the variation will be sufficiently minimized if strict standardization in the preparation of test samples is introduced.

The most remarkable advantage of the present approach based on SPR immunosensor over the IBDV diagnostic strip lies in its ability of quantitative detection of IBDV. In comparison with the immunofluorescence test, western blot, enzyme-linked immunosorbent assay, rocket immunoelectrophoresis and real-time polymerase chain reaction method, this approach comes with significant benefits in being time saving and labeling-free. The assay time required is no more than 30 min if the IBDV biochips have been prepared in advance. The proposed SPR biosensor was sufficiently selective for detecting IBDV from infected chicken tissues. The detection limit of SPR biosensor biochip is 1/18 of that of the IBDV diagnostic strips.

4. Conclusion

In this work, an innovative, specific and label-free detection approach based on SPR biosensor and the corresponding measuring procedures have been developed for the detection of IBDV. Very satisfactory detection of IBDV from chicken tissue sample was achieved, with the detection limit as low as 2.5 ng/ml. This approach manifested obvious advantages over other approaches of similar applications, such as no need for molecular labeling, nondestructive quantification of analyte, light weight and small size, which give the approach clear potential in the development of portable, low-cost and simple devices for detecting IBDV. Such devices will help to promote effective implementation of sound control strategies against IBDV. Future work is desired in the following aspects: standardization of sample preparation to minimize the variation in the background RUs among different samples, tests of a large number of samples of both non-infected and infected chickens for the quantification of variations of the background RUs and the recovery rates, and the development of a statistical model for the probabilistic identification of IBDV infection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.11.019.

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