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Development and evaluation of surface plasmon resonance imaging for the detection of antibodies against classical swine fever virus in swine

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Abstracts

A protein chip based on surface plasmon resonance (SPR) imaging was developed for measuring classical swine fever virus (CSFV) antibody using a recombinant gp55 protein as an antigen. The diagnostic potential of SPR imaging for detecting antibodies to the CSFV gp55 protein was compared with that of a enzyme-linked immunosorbent assay (ELISA) using 70 pig sera. There was a strong positive correlation between the SPR imaging and ELISA ($n = 70$, $r = 0.916$, $p < 0.01$). Therefore, the SPR imaging, which is a label-free and high-through put method, is expected to be a valuable tool in the serodiagnosis of CSFV.

Key words: Classical swine fever, Protein chip, Surface plasmon resonance imaging

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Introduction

Classical swine fever (CSF) is a highly contagious and often fatal disease in swine, affecting both the domestic and wild pig population[1)]. Classical swine fever virus (CSFV) is the causative agent of CSF, and is a member of the genus *Pestivirus*, which belongs to the family *Flaviviridae*[2)].

A specific and sensitive serological test for the detection of antibodies (Abs) to CSFV is needed for the surveillance and diagnosis of CSF[3, 4)]. There is need of development of more sensitive and specific assay for the detection of CSFV

which can identify the animals infected within herd before it starts shedding of virus. Such test plays significant role in epidemiological and clinical applications [5, 6]. Various methods have been developed to detect Abs against CSFV such as virus neutralization test or antibody (Ab) ELISA [3, 5-7]. Although these methods are quite reliable, they are time-and labor-intensive protocols. Most protein arrays currently developed rely on detection technologies that employ enzymatic or fluorescent tags. In contrast, Surface plasmon resonance (SPR) as a label-free technology for monitoring biomolecular interactions is a promising technique for rapid and parallel detection [8]. SPR imaging using fast optical array detectors permits simultaneous measurements across an array of immobilized molecules. SPR imaging provides excellent spatial resolution at the same sensitivity as classical SPR. These features make SPR imaging a promising detection technology for biochips [9].

In this study, the recombinant gp55 protein [10] was used to develop a protein chip based on SPR imaging for measuring the Abs of CSFV in pig sera. The diagnostic efficacy of SPR imaging in determining the CSFV infection and screening the Ab titers after vaccination was compared with that of conventional ELISA.

Materials and Methods

A recombinant gp55 protein was prepared and a monoclonal Ab was prepared as described previously [10]. A total of 70 pig serum samples were obtained randomly from both genders (various breeds and ages) at 6 pig farms (an average of 12 serum samples per farm). The sera were prepared with 1 : 20 dilutions and used for ELISA and SPR.

All samples were analyzed for any reactivity against CSFV antigen by using a commercial ELISA kit (Jeno Biotech Inc, Chuncheon, Korea). The assays were performed according to the manufacturer's instructions. The optical density (OD) of the positive control was 0.5 and the OD of the negative control was 0.3. To validate the ELISA results, the value of a corrected positive control (CPC) was 0.3 (CPC = mean OD of positive control mean OD of negative control). The ELISA results were analyzed by calculating the sample to positive ratio (S/P ratio) of a sample using the following formula, S/P ratio = (OD of sample OD of NC) / CPC. Based on the S/P ratio, 0.14 was considered positive and < 0.14 was considered negative. As the control for SPR imaging, bovine viral diarrhea virus monoclonal Ab (Jeno Biotech Inc, Chunchon, Korea), porcine circovirus type 2 monoclonal Ab (Jeno Biotech Inc, Chuncheon, Korea) and normal porcine sera were used in this study.

The surface modification of a gold chip for the specific binding of antigen was carried out as described elsewhere [10]. Briefly, a patterned glass slide chip with a gold film (K-Mac, Daejeon, Korea) was used to prepare the gold chip. Prior to coating with ProLinker B (Proteogen, Seoul, Korea), the gold-coated glass slides were cleaned using a freshly prepared piranha solution (3 : 1 mixture of concentrated H_2SO_4 and 30% H_2O_2). After washing with deionized water, the glass

slides were dried under a N_2 stream. The gold chip was prepared by soaking a clean gold-coated chip in 3 mM ProLinker B solution for 1 hr followed by rinsing sequentially with $CHCl_3$, acetone, ethanol, deionized water and drying. For quantitative assays of Abs with a minute amount of serum samples (1 ㎕ per well), the antigens in phosphate buffered saline (PBS, pH 7.4) were spotted onto the ProLinker B coated gold chip using the ProteoChip (Proteogen, Seoul, Korea) with miniaturized micro-wells. After the spotting, the chip was incubated for 20 min in 80% humidity at 37℃. The spotted chip was rinsed three times with phosphate buffered saline tween-20 (PBST), followed by rinsing with deionized water. After adding 1% bovine serum albumin (BSA) in PBS for 10 minutes to block the chip surface, the solution containing the Ab or serum samples in a PBS buffer containing 0.1 ㎎/㎖ BSA was applied to the chip for 15 min. The chip was then dried and analyzed using the SPR imaging system (SPRi LAB, K-MAC, Daejeon, Korea). The detection limit was determined by examining serial dilutions of the monoclonal anti-CSFV (gp55) Ab (Jeno Biotech Inc., Chuncheon, Korea). The concentrations used were 10 fold dilutions ranging from 0.1 ㎎/㎖ to 1 ng/㎖.

The correlation coefficients between ELISA and SPR imaging were calculated. A p value < 0.01 was considered significant. The Pearson's correlation coefficient (SPSS Base 12.0, SPSS, Chicago, IL, USA) was used to determine the strength of the association between the SPR imaging and ELISA.

Results and Discussion

ELISA was used to determine CSFV specific Abs in pig sera. As a result, 70 samples (100%) were positive. The sample to positive ratio (S/P ratio) at least 0.14 above negative controls was considered as positive. The specificity was confirmed by performing SPR imaging from monoclonal Abs of bovine viral diarrhea virus and porcine circovirus type 2 (PCV2) (Jeno Biotech Inc. Chuncheon, Korea) and normal porcine sera. The signal intensity for SPR imahging was not detected.

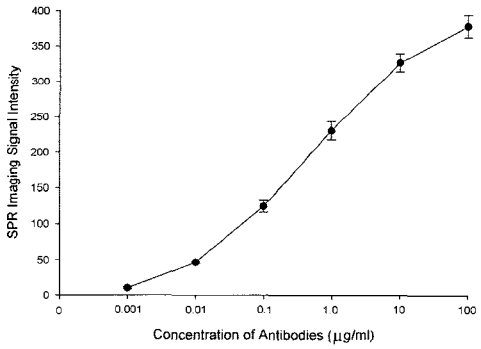

Fig 1. The changes in the surface plasmon resonance imaging signal intensity due to binding between the antigen (gp55) immobilized Prolinker B and various dilutions of control antibody [monoclonal anti-classical swine fever virus (gp55)].

Fig 1 shows the relationship between the signal intensity in the SPR imaging as a function of the positive control Ab dilution. The figure shows that the signal intensity in the SPRimaging increased linearly with increasing Ab concentration. The detection limit of the SPR protein chip corresponded to a 10^{-2} g/㎖ Ab concentration, which corresponded to a signal intensity of 51.3

(mean = 46.2, SD = 1.31). All 70 samples (100%) also tested positive by SPR imaging, suggesting that a chip based on SPR imaging was as sensitive as ELISA.

Fig 2 shows the signal intensity of the 70 serum samples. Mouse anti-CSFV gp55 Ab (positive control) and "no primary Ab" (negative control) were used in the SPR imaging, which required only 1 hour. There was a good correlation between SPR imaging signal intensity and ELISA S/Pvalues for the 70 pigs serum samples ($r = 0.916$, $p < 0.01$) (Fig 3).

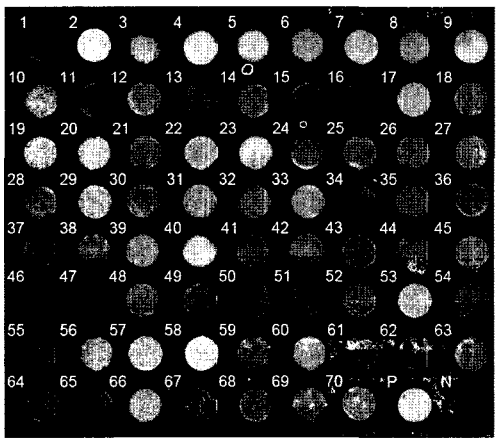

Fig 2. Surface plasmon resonance (SPR) imaging analysis of the classical swine fever virus antibodies (gp55 as an antigen) for 70 pigs serum samples. 1-70: serum sample number, P: positive control, N: negative control.

The newly developed SPR imaging was used successfully to detect anti-CSFV gp55 Abs in pigs sera. This comparative study showed that although ELISA is quite reliable, it is time-consuming. Recently, several SPR assays have been described for detection of Abs against pathogens such as PCV2 [11], CSFV[1], and *Mycoplasma hyopneumoniae* [12]. However, the SPR assay can only be applied to 2 to 4 samples simultaneously. Compared with the SPR assay, the newly developed SPR imaging protein chip with miniaturized micro-wells proved to be a rapid (assay time 1 hour), high-throughput, and valuable tool for the serodiagnosis of CSFV infection and Ab titers after vaccination.

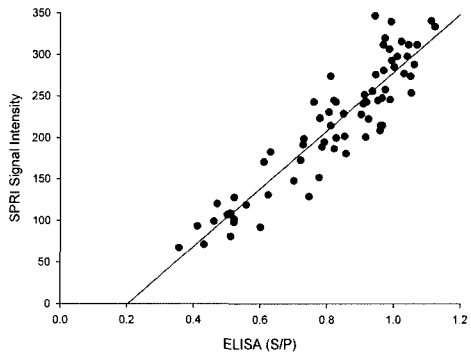

Fig 3. Correlation between the antibody optical density values of the classical swine fever virus in the swine sera determined by ELISA and signal intensity determined by surface plasmon resonance imaging (Pearson correlation coefficient n = 70, r = 0.916, $p < 0.01$).

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