



Surface-displayed porcine reproductive and respiratory syndrome virus from cell culture onto gram-positive enhancer matrix particles

Lan Li¹ · Xuwen Qiao¹ · Jin Chen¹ · Yuanpeng Zhang¹ · Qisheng Zheng¹ · Jibo Hou^{1,2}

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Abstract

Vaccine immunization is now one of the most effective ways to control porcine reproductive and respiratory syndrome virus (PRRSV) infection. Impurity is one of the main factors affecting vaccine safety and efficacy. Here we present a novel innovative PRRSV purification approach based on surface display technology. First, a bifunctional protein PA-GRFT (protein anchor-griffithsin), the crucial factor in the purification process, was successfully produced in *Escherichia coli* yielding 80 mg/L of broth culture. Then PRRSV purification was performed by incubation of PA-GRFT with PRRSV and gram-positive enhancer matrix (GEM) particles, followed by centrifugation to collect virions loaded onto GEM particles. Our results showed that most of the bulk impurities had been removed, and PA-GRFT could capture PRRSV onto GEM particles. Our lactic acid bacteria-based purification method, which is promising as ease of operation, low cost and easy to scale-up, may represent a candidate method for the large-scale purification of this virus for vaccine production.

Keywords Lactic acid bacteria · GEM particles · Purification · PRRSV · Griffithsin

The authors, Lan Li and Xuwen Qiao contributed equally as first authors.

✉ Qisheng Zheng
njcvc1302@163.com

✉ Jibo Hou
houjibocvv@163.com

Lan Li
lanli@foxmail.com

Xuwen Qiao
qxwshyi2011@hotmail.com

Jin Chen
chenjin_abc@163.com

Yuanpeng Zhang
yuanpengzhangnj@163.com

¹ National Research, Center of Engineering and Technology for Veterinary Biologicals, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China

² Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, Jiangsu, China

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection has been causing significant economic losses in the swine industry worldwide [17]. PRRSV is an enveloped, positive sense, single-stranded RNA virus. The 15.4-kb genome encodes at least ten open reading frames [4] including four envelope glycoproteins designated GP2a, GP3, GP4 and GP5, a nonglycosylated membrane protein M and the most abundant nucleocapsid protein N [12]. The major envelope glycoprotein GP5 is disulfide-linked to M protein and the GP5/M heterodimers are likely to form the basic protein matrix of the virion envelope [14].

Currently, vaccination is the most prevalent measure to control PRRSV infection. The purification of viruses is essential prior to use in prophylactic vaccines because cellular and media contaminants can induce toxicity, inflammation and immune responses [8]. Specifically, ultracentrifugation, ultrafiltration and chromatography methods have been used for PRRSV purification [1, 8]. However, these methods do not currently allow for the production of large quantities of purified virus.

The display of heterologous proteins on the surface of lactic acid bacteria is gaining increasing attention in various fields of biotechnology, including separation technologies

and vaccine delivery [22]. Proteins can be attached to GEM particles, lactococcal-derived bacterial-shaped particles by PA [22]. These GEM particles are non-living and non-genetically modified spherical particles composed of the cell wall. The GEM particles, which are deprived of intact surface proteins and intracellular content [2], are characterized by improved activity, stability and preservation. Another advantage is that GEM particles reduce the risk of dispersion of recombinant DNA into the environment [15]. One previously reported protein anchor for surface display is the lysin motif (LysM). LysM has been discovered in various proteins, such as bacterial lysins, bacteriophage proteins and certain proteins of eukaryotes [3]. The LysM used in this study originates from the C-terminal peptidoglycan-binding domain of AcmA, an autolysin from *Lactococcus lactis*, and it binds with high affinity to GEM particles in a non-covalent manner [22]. This novel surface display system offers the possibility to scale-up the virus purification process.

Binding of glycans to multiple sites on a single molecule of griffithsin (GRFT), isolated from the marine red alga *Griffithsia* sp. [16], provides the basis for our virus purification strategy. GRFT, a 13-kDa lectin, was recently shown to tightly bind to the glycoprotein on the surface of a number of enveloped viruses including human immunodeficiency virus [5, 30, 33], severe acute respiratory syndrome corona virus [28], hepatitis C virus [21], herpes simplex virus 2 [27], Japanese encephalitis virus [9], Middle East respiratory syndrome coronavirus [23] and human papillomavirus [13]. However, there are limited data available regarding the binding activities of GRFT to viruses in the veterinary field. Besides, GRFT shows superior stability, remaining functionally stable at 80 °C [5], and when exposed to repeated lyophilization [25], organic solvents and proteases [24]. Researchers have confirmed that GRFT has an outstanding safety profile with little toxicity or deleterious immunological consequences [10, 11], which provides further evidence in support of the clinical application of GRFT.

In the present study, we designed a bifunctional protein PA-GRFT and developed a novel approach for PRRS virus purification as shown in Fig. 1. In detail, PA-GRFT was successfully produced in *Escherichia coli* yielding 80 mg/L of broth culture and its expected function (i.e., glycans and GEM binding activity) was confirmed. After incubation of PA-GRFT with PRRSV and GEM particles, one-step low-speed centrifugation was carried out to obtain purified PRRSV. Western blotting, SDS-PAGE, immunofluorescence microscopy and TCID₅₀ analysis showed that the PA-GRFT partnership could direct PRRSV onto the GEM surface with satisfactory efficiency.

Methods

Bacterial strains, plasmids and culture conditions

The bacterial strains and plasmids used in this study were as follows. *Lactococcus lactis* MG1363 (China Committee for Culture Collection of Microorganisms, China) was grown in M17 broth (Merck, Germany) supplemented with 1% glucose (GM17) at 30 °C in standing cultures. The *E. coli* strains (TaKaRa, Japan) were grown aerobically in Luria–Bertani (LB) broth at 37 °C. For the selection of transformants in *E. coli* strains, ampicillin and chloramphenicol were used to supplement the media at 100 and 20 µg/mL, respectively. Plasmid PA3 was constructed by our colleague Dr Pengcheng Li at National Research Center of Engineering and Technology for Veterinary Biologicals, Jiangsu Academy of Agricultural Science.

Cells and viruses

MARC-145 cells were grown in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% newborn bovine serum at 37 °C and 5% CO₂. HP PRRSV strain NJ-a (type 2 PRRSV) was isolated, identified and stored in our laboratory at National Research Center of Engineering and Technology for Veterinary Biologicals, Jiangsu Academy of Agricultural Science. NJ-a strain was propagated in MARC-145 cells. The virus stock was clarified by low-speed centrifugation at 5000×g for 20 min at 4 °C after three consecutive freeze/thaw cycles of the infected cells. The virus stock titer was 10^{8.0} TCID₅₀/mL in this study.

DNA manipulation and the construction of recombinant *E. coli* strains

The *griffithsin* gene (GenBank: AY744143), with *Nde*I and *Bam*HI restriction sites added to the 5' and 3' ends, was codon-optimized for *E. coli* expression, synthesized and cloned into the pUC57 vector using GenScript (Nanjing, China). The *PA* gene, with *Bam*HI and *Xho*I restriction sites, was amplified from the plasmid PA3 and cloned into pMD19-T vector, which was selected with ampicillin. The GRFT fragment digested with *Nde*I–*Bam*HI and the *PA* fragment digested with *Bam*HI–*Xho*I were introduced into the *Nde*I–*Xho*I digested pET-32a vector to obtain the recombinant expression plasmid pET-32a-PA-GRFT. Double-enzyme cleavage of the recombinant expression plasmid was used to verify the gene insertion. The recombinant expression plasmid was then transformed into competent *E. coli* BL21 (DE3) cells using the heat-shock technique resulting in the recombinant *E. coli* BL21 pET32a-PA-GRFT strain,

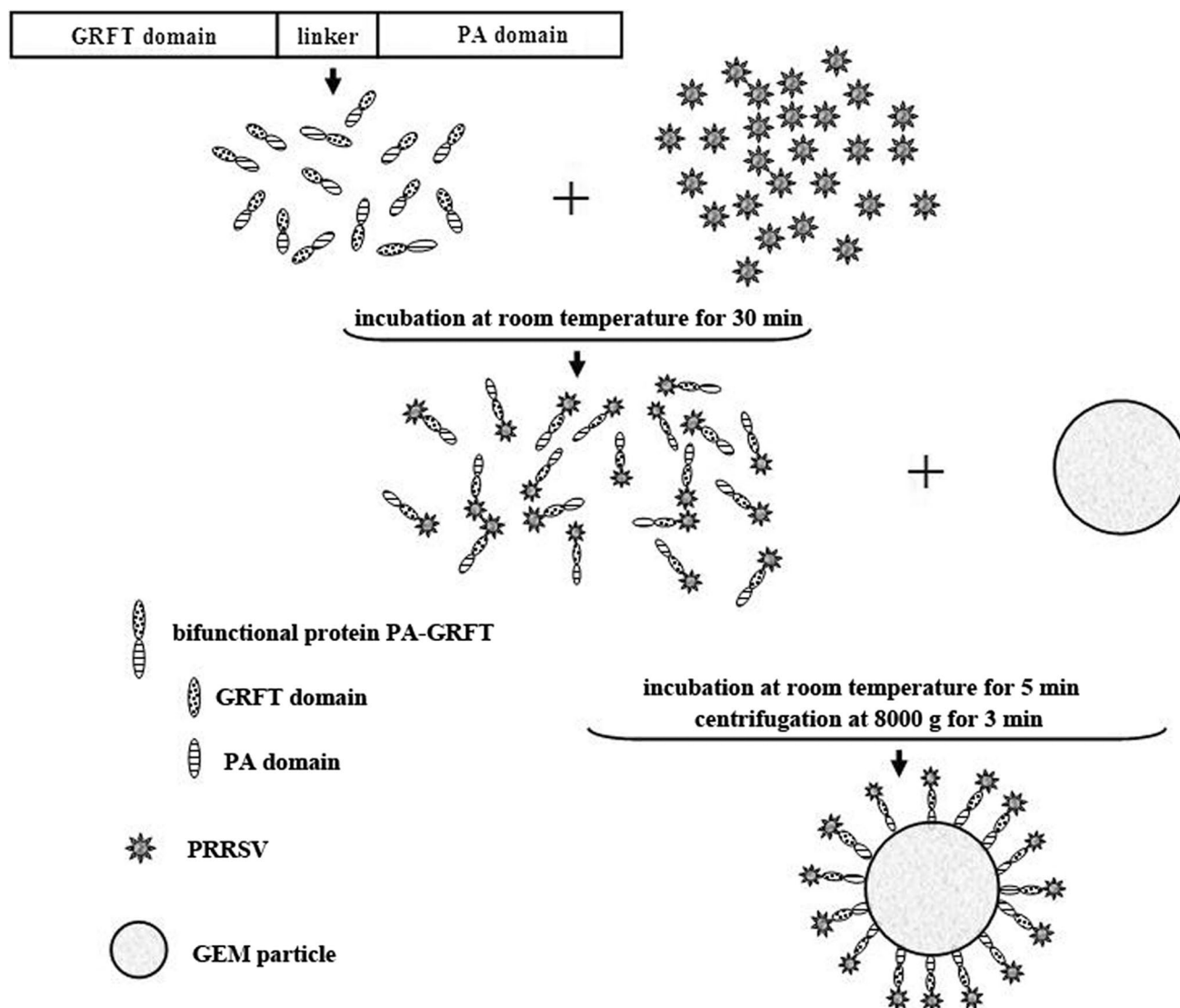


Fig. 1 Schematic representation illustrating the process of GEM particle displaying PRRSV by PA-GRFT

which was selected by ampicillin and chloramphenicol. Concurrently, pET-32a was transformed into competent *E. coli* BL21 (DE3) cells resulting in an *E. coli* BL21 pET32a strain as a negative control.

Bifunctional anchoring protein expression in *E. coli* BL21 (DE3)

Gene expression was carried out as described in the pET system manual (Novagen, USA) in *E. coli* BL21 (DE3) cells. Recombinant *E. coli* strains were grown overnight at 37 °C in LB broth containing 100 µg/mL ampicillin and 20 µg/mL chloramphenicol. Subsequently, the overnight cultures were 100-fold diluted in 400 mL of fresh LB broth at 37 °C and induced for protein expression with 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) when the cultures reached

an OD₆₀₀ of 1.0. After further incubation for 24 h at 15 °C, the culture was harvested by centrifugation at 6000×g for 5 min, washed twice and resuspended in 80 mL of PBS. Then the cells were disrupted by high-pressure homogenization for two cycles. Finally, the cell lysates were centrifuged at 12,000×g for 20 min at 4 °C and the resulting supernatants named as the crude PA-GRFT extract were stored at –20 °C until use. SDS-PAGE and western blotting were used to analyze protein expression. In addition, PA-GRFT in the crude extract was quantified by ELISA as follows.

OVA-binding activity of PA-GRFT via the lectin domain

Binding of PA-GRFT to ovalbumin (OVA) was assessed as previously described with some modifications [29, 31].

Briefly, 10 µg of OVA was coated onto each well in a 96-well plate overnight at 4 °C. The plates were rinsed with 0.1% Tween 20 in PBS and blocked with 1% BSA for 3 h at 37 °C. Serial dilutions of the crude PA-GRFT extract alone were then added to the wells in triplicate and incubated for 1 h at 37 °C. In competition assays, dilutions of the crude PA-GRFT extract were pre-incubated for 30 min at room temperature with OVA and then this mixture was added to the wells; the crude extract containing no PA-GRFT was used as a negative control. For PA-GRFT detection, anti-His tag antibody diluted 1:2000 with 1% BSA in PBS and HRP-conjugated secondary antibody (Boster, Wuhan, China) diluted 1:3000 in BSA-PBS were used. For horseradish peroxidase detection, TMB was added to each well and after 15 min at 37 °C the reaction was stopped with 2 M H₂SO₄ and the absorbance at 450 nm was read.

GEM particle preparation

Lactococcus lactis MG1363 was cultured overnight. Then cells were collected and washed once with sterile distilled water. Then cells were resuspended in a 0.2 volume of 0.1 M hydrochloric acid (pH 1.0) and boiled for 30 min. Subsequently the GEM particles were pelleted and washed completely with PBS. Finally, the GEM particles were resuspended in PBS and stored at –80 °C until use. One unit (U) of GEM particles was defined as 2.5×10^9 nonliving particles.

Binding conditions of PA-GRFT by PA domain to GEM particles

Different amounts of PA-GRFT were incubated with 1 U of GEM particles at room temperature for 30 min to optimize the quantity ratio of PA-GRFT and GEM particles, and the crude extract containing no PA-GRFT was used as a negative control. After binding, the PA-GRFT loaded onto GEM particles was collected by centrifugation at 8000×g for 3 min and rinsed twice with PBS for SDS-PAGE detection. Also the supernatants were collected for ELISA analysis to verify the maximum binding amount of 1 U of GEM particles to PA-GRFT.

The reaction time between PA-GRFT and the GEM particles was also studied by incubating crude PA-GRFT extract with 1 U of GEM particles at room temperature for 1, 3 and 5 min, respectively. After centrifugation, the samples were subjected to SDS-PAGE detection.

Surface-displayed PRRSV from cell culture onto GEM particles via PA-GRFT

Initially, the crude extract containing 200 µg of PA-GRFT or not was incubated with different amounts of the virus

stock at room temperature for 30 min and then 2 U GEM particles were added and incubated for 5 min. Only virus incubated with GEM particles was also used as a negative control. After binding, virions loaded onto GEM particles by PA-GRFT were obtained by centrifugation at 8000×g for 3 min and three washes with PBS. The virions and supernatants were collected for further detection. To assess the efficiency of PA-GRFT in directing the virions to the GEM surface, western blotting, SDS-PAGE, immunofluorescence microscopy and TCID₅₀ determination were used.

SDS-PAGE and western blotting analysis

Samples were loaded onto 15% SDS-PAGE, transferred to PVDF membrane and blocked with 5% skim milk in TBST buffer overnight. Membranes were then incubated with mouse anti-PRRSV GP5 protein monoclonal antibody (Ab0073, TONGDIAN, Hangzhou, China) or swine PRRSV positive sera. After 1 h, membranes were incubated with an appropriate secondary antibody (Boster, Wuhan, China) for 1 h at 37 °C. The bands were visualized using an enhanced chemiluminescence (ECL) reagent and recorded with an ImageQuant LAS 4000 (GE Healthcare, Sweden).

Immunofluorescence microscopy

The capacity of PA-GRFT to direct the virions to the GEM particles was further confirmed by immunofluorescence microscopy. Briefly, the virions loaded onto the GEM particles were suspended in PBS containing 1% BSA and then incubated with anti-PRRSV GP5 protein monoclonal antibody diluted 1:1000 with BSA-PBS for 1 h at 37 °C. After washing three times with PBS, the suspension was incubated with FITC-conjugated goat anti-mouse IgG antibody (Boster, Wuhan, China) diluted 1:30 for 1 h at 37 °C in the dark. After washing, the sample was spread onto a polysin microslide and examined by laser scanning confocal microscopy.

TCID₅₀ detection

Virus titer of PRRSV was performed using MARC-145 cells in 96-well plates. Samples including the supernatant and virus stock were serially tenfold diluted in DMEM medium without newborn bovine serum. Six wells were inoculated with 100 µL at each dilution. Cells continued to be cultured at 37 °C for 1 h and thereafter the cells were washed with PBS three times. Then DMEM medium with 2% new born bovine serum was added and the cells incubated for 5 days until a cytopathic effect appeared. TCID₅₀ calculation was based on the Reed Muench formula. PRRS virus recovery was calculated using the following formula:

$$\text{recovery rate (\%)} = 1 - \frac{\text{number of virus}_{\text{supernatant}}}{\text{number of virus}_{\text{virus stock}}}$$

Results

Design and expression of the bifunctional protein

We designed a bifunctional protein in which the lectin domain and the PA domain were combined by a flexible and unstructured linker. The sequence of PA-GRFT thus comprised amino acids 1–121, which encoded the GRFT domain, followed by a linker (SSSGGGSGGGSSSGSGS), and residues 140–260, which encoded the PA domain.

The resulting expression protein was expressed as a C-terminally His-tagged protein and was analyzed using SDS-PAGE and western blotting as shown in Fig. 2. Soluble PA-GRFT (27 kDa) was successfully obtained in *E. coli* BL21 (Fig. 2a), and a 27-kDa protein band was detected by western blotting using an anti-His antibody (Fig. 2b). Furthermore, the concentration of PA-GRFT in the crude extract was around 400 $\mu\text{g/mL}$ compared with a standard curve of purified PA-GRFT using Ni^{2+} affinity column, ranging from 12.5 to 200 $\mu\text{g/mL}$. Thus a yield of 80 mg of soluble PA-GRFT per L of broth culture was obtained.

OVA-binding activity of PA-GRFT via the lectin domain

To determine whether PA-GRFT could bind to glycans on protein surfaces, the amount of PA-GRFT bound to OVA-coated wells was measured by ELISA. OVA contains a single *N*-linked glycosylation site, in which high-mannose and hybrid *N*-linked glycans have been characterized. Figure 3 shows that PA-GRFT bound to OVA in a dose-dependent manner, with background levels of binding only evident

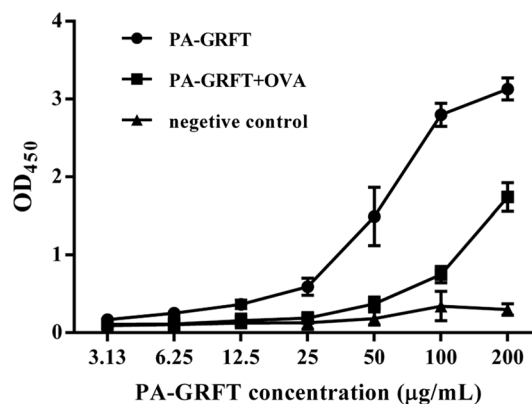


Fig. 3 Binding of PA-GRFT to OVA-coated wells and detection by ELISA

in the negative control. The amount of PA-GRFT was significantly reduced at concentrations of 100 and 200 $\mu\text{g/mL}$ when PA-GRFT was pre-incubated with OVA.

GEM-binding activity of PA-GRFT via the PA domain

After incubation with the crude extract containing 25, 50, 100, 120, 150, and 180 μg of PA-GRFT, respectively, the loaded GEM particles were used for SDS-PAGE analysis. As shown in Fig. 4, PA-GRFT anchored to the GEM particles exhibited a specific protein band. By contrast, when 1 U of GEM particles was saturated with 120 μg of PA-GRFT, no PA-GRFT was found in the supernatant, whereas when more than 120 μg of PA-GRFT was added some proteins remained in the supernatant after binding detected by ELISA assay. We next attempted to investigate the reaction time between GEM particles and PA-GRFT; the data showed that the reaction was extremely rapid, being complete within 1 min (Fig. 5).

Fig. 2 Analysis of PA-GRFT expression. **a** SDS-PAGE stained with Coomassie Brilliant Blue. **b** Western blotting analysis using the anti-His tag antibody. **a, b** Lane M, protein size markers; Lane 1, supernatant of *E. coli* BL21 pET32a-PA-GRFT after homogenization; Lane 2, sediment of *E. coli* BL21 pET32a-PA-GRFT after homogenization; Lane 3, supernatant of *E. coli* BL21 pET32a after homogenization; Lane 4, sediment of *E. coli* BL21 pET32a after homogenization

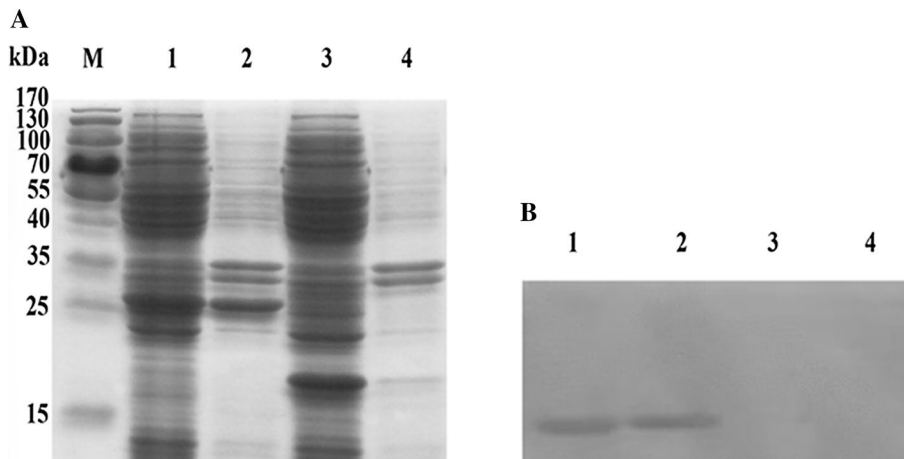


Fig. 4 SDS-PAGE analysis of binding efficiency of PA-GRFT to GEM particles. Lane M protein size markers; Lane 1 the crude PA-GRFT extract; Lane 2 negative control, the crude extract containing no PA-GRFT incubated with GEM particles; Lanes 3–8, PA-GRFT loaded onto GEM particles 25, 50, 100, 120, 150 and 180 μg of the crude PA-GRFT extract incubated with GEM particles, respectively

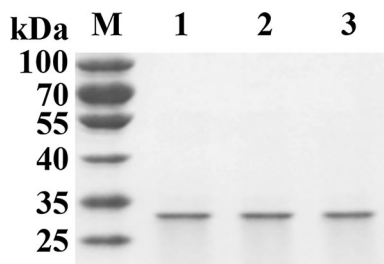
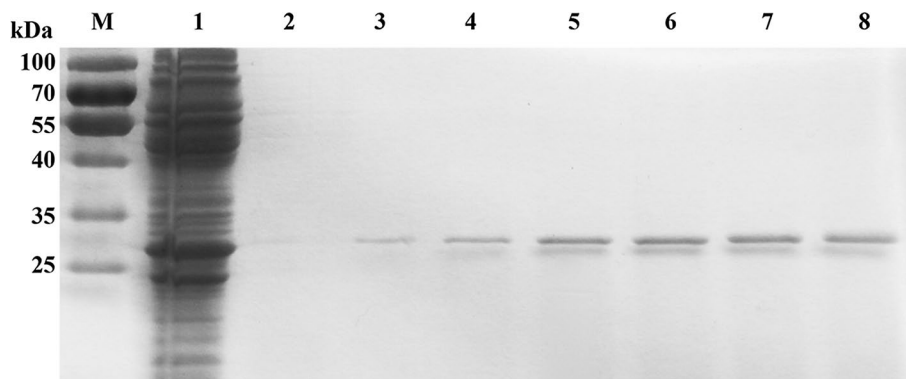


Fig. 5 SDS-PAGE analysis of the reaction time between GEM particles and PA-GRFT. Lane M, protein size markers; Lanes 1–3 PA-GRFT loaded onto GEM particles, the crude PA-GRFT extract incubated with GEM particles for 1, 3 and 5 min, respectively

Surface-displayed PRRSV from cell culture onto GEM particles by PA-GRFT

PRRSV particles were purified by a one-step low-speed centrifugation. Data showed that the GP5 proteins were enriched on the GEM particles incubated with PA-GRFT and still present in the supernatants without PA-GRFT in the negative control groups (Fig. 6a), which indicated that PA-GRFT could capture PRRSV onto the GEM particles as we had expected. Furthermore, as shown in Fig. 6a, no detectable GP5 proteins were present in the supernatants when less than 50 mL of virus stock was added, which is the maximum purification capacity of 200 μg of PA-GRFT with 2 U GEM particles. Besides, PRRSV loaded onto the GEM particles presented a 27-kDa protein band on Coomassie-stained SDS-PAGE corresponding to PA-GRFT, which was much thinner than the most prominent band of virus stock. And the band at 27 kDa was not detected in the negative control groups (Fig. 6b). Except the PA-GRFT band, few faint bands corresponding to the media and cellular proteins could be detected even when the sample was 20-fold concentrated (Fig. 6b). Thus most of the bulk impurities had been removed and the viruses loaded onto the GEM particles were highly pure following purification. In addition, immunofluorescence microscopy was performed and microscopic observations indicated PRRSV loaded onto GEM particles

by PA-GRFT displayed strong green fluorescence as shown in Fig. 7a, and there were no green fluorescence in the negative controls (Fig. 7b, c).

Western blotting analysis was also performed using swine PRRSV positive sera, and the results was illustrated by Fig. 8. Purified PRRSV (Lane 2) showed two major protein bands, N protein appeared at 14 kDa and GP5 protein appeared at 25 kDa. GP3 protein and minor proteins such as GP2a, GP3 and GP4 were not visible probably due to low abundance. Besides, there was a band at approximately 50 kDa corresponding to protein impurity from the media or cellular protein, probably caused by non-specific adsorption of GEM particles.

To further confirm the purification efficiency of PA-GRFT on the GEM surface display platform, TCID_{50} of the supernatant and virus stock was detected and shown in Fig. 9. The titer of supernatants was $10^{3.5}$, $10^{3.9}$ and $10^{3.1}$ $\text{TCID}_{50}/\text{mL}$ when 30, 40 and 50 mL of virus stock was added, which showed significant reduction compared with the virus stock $10^{8.0}$ $\text{TCID}_{50}/\text{mL}$ indicating PRRSV was attached onto GEM particles. However, the titer of supernatants was $10^{6.6}$ $\text{TCID}_{50}/\text{mL}$ when 60 mL of virus stock was added. Thus 200 μg of PA-GRFT with 2 U GEM particles could purify 50 mL of virus stock, which is consistent with the western blotting analysis. Particularly recovery rate can reach 99.9% calculated according to TCID_{50} .

Discussion

Vaccine immunization is now one of the most effective ways to control PRRSV infection. The demand for high-quality vaccine that is safer and more efficient is increasing. Impurity is one of the main factors affecting vaccine safety and efficacy. The purification of viruses has become increasingly important for vaccine development and improved methods of purification are, therefore, highly desired. For this reason, we sought to develop an approach for PRRSV purification. We found that PRRSV could be properly and efficiently attached to the surface of GEM particles. The purification

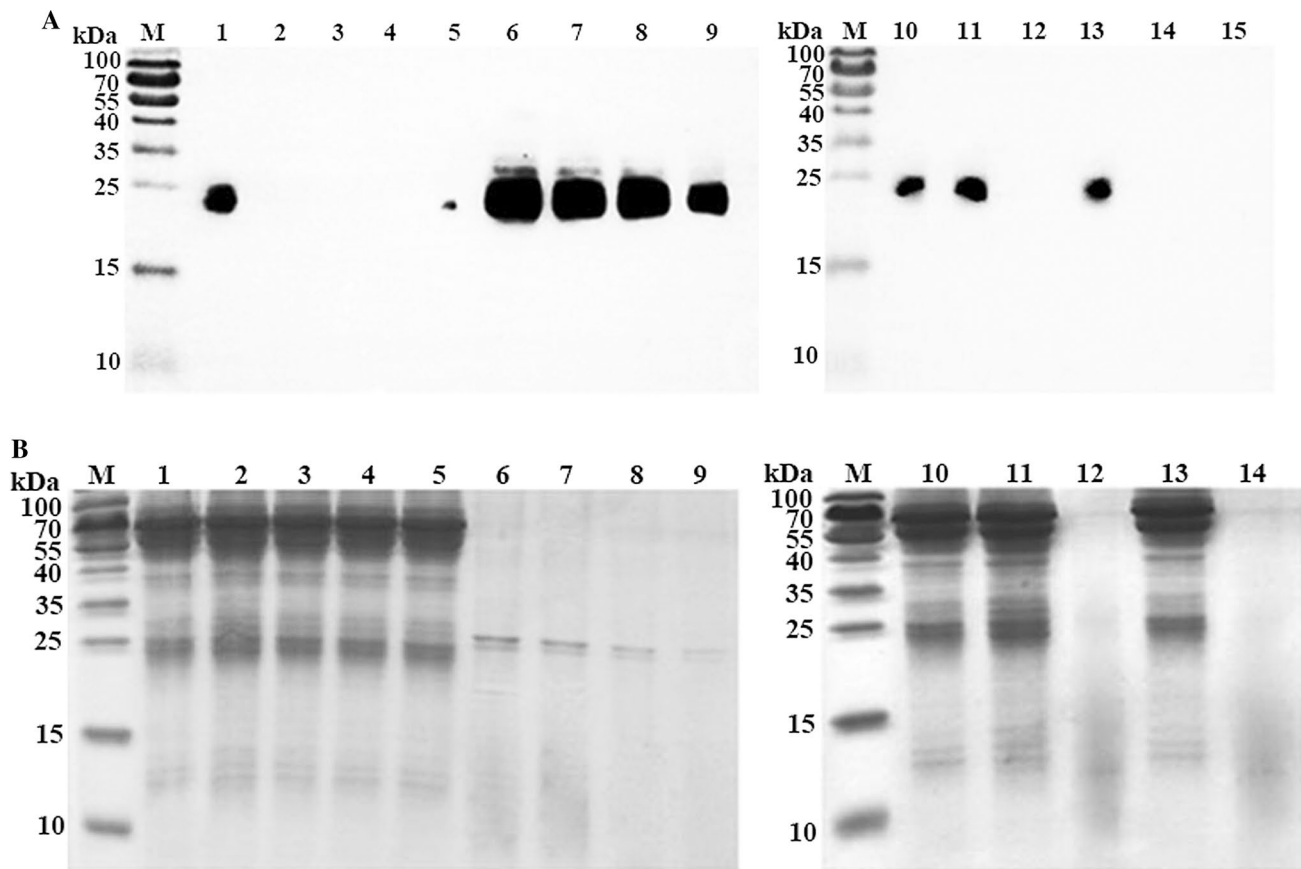


Fig. 6 Analysis of the separation of PRRSV from cell culture onto the GEM particles. **a** Western blotting detection using anti-PRRSV GP5 protein monoclonal antibody. **b** SDS-PAGE analysis stained with Coomassie Brilliant Blue. Lane M, protein size markers; Lanes 1, 10 virus stock; Lanes 2–5, supernatant corresponding to 30, 40, 50 and 60 mL of virus purification by the crude PA-GRFT extract; Lanes 6–9, PRRSV loaded onto GEM particles corresponding to 30, 40, 50 and 60 mL of virus purification by the crude PA-GRFT extract; Lane

11, supernatant corresponding to 30 mL of virus purification by the crude extract containing no PA-GRFT; Lane 12, PRRSV loaded onto GEM particles corresponding to 30 mL of virus purification by the crude extract containing no PA-GRFT; Lane 13, supernatant corresponding to 30 mL of virus purification incubated with GEM particles; Lane 14, PRRSV loaded onto GEM particles corresponding to 30 mL of virus purification incubated with GEM particles; Lane 15, the crude PA-GRFT extract

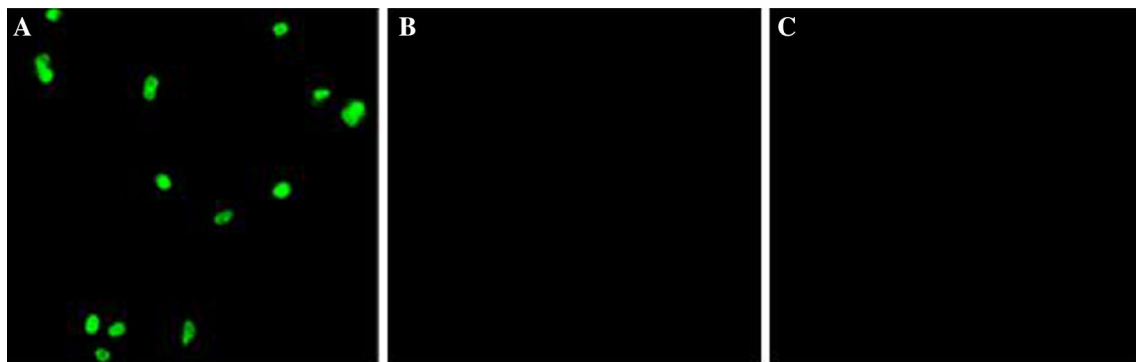


Fig. 7 Detection of PRRSV on the surface of GEM particles by immunofluorescence microscopy. **a** PRRSV loaded onto GEM particles by the crude PA-GRFT extract. **b** PRRSV loaded onto GEM particles by the crude extract containing no PA-GRFT. **c** PRRSV loaded onto GEM particles incubated with GEM particles

ticles by the crude extract containing no PA-GRFT. **c** PRRSV loaded onto GEM particles incubated with GEM particles

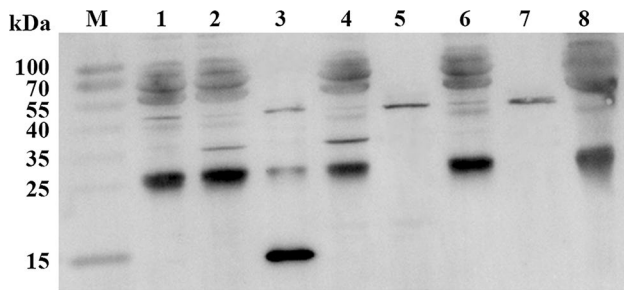


Fig. 8 Western blotting detection using swine PRRSV positive sera. Lane M, protein size markers; Lane 1, virus stock; Lane 2, supernatant corresponding to 50 mL of virus purification by the crude PA-GRFT extract; Lane 3, PRRSV loaded onto GEM particles corresponding to 50 mL of virus purification by the crude PA-GRFT extract; Lane 4, supernatant corresponding to 50 mL of virus purification by the crude extract containing no PA-GRFT; Lane 5, PRRSV loaded onto GEM particles corresponding to 50 mL of virus purification by the crude extract containing no PA-GRFT; Lane 6, supernatant corresponding to 50 mL of virus purification incubated with GEM particles; Lane 7, PRRSV loaded onto GEM particles corresponding to 50 mL of virus purification incubated with GEM particles; Lane 8, the non-infected MARC-145 cells

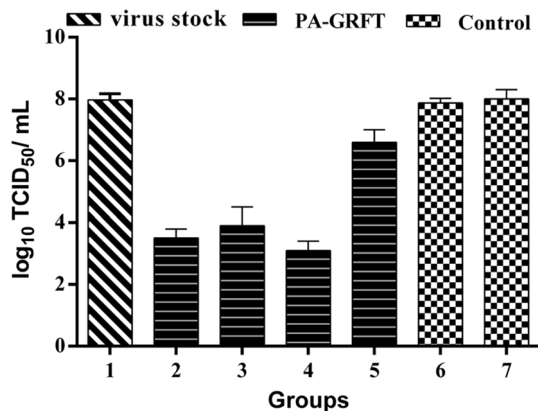


Fig. 9 Determination of TCID₅₀ of supernatant after virus purification and virus stock by an end-point dilution assay on MARC-145 cells. 1 virus stock, 2–5 supernatant corresponding to 30, 40, 50 and 60 mL of virus purification by the crude PA-GRFT extract; 6. supernatant corresponding to 30 mL of virus purification by the crude extract containing no PA-GRFT; 7 supernatant corresponding to 30 mL of virus purification incubated with GEM particles

process employed was easy to perform, cost efficient and scalable and may, therefore, be superior compared with other previously established preparative purification methods [1, 8]. Thus, our approach involving the separation of PRRSV from the cell culture onto GEM particles may be a promising method for large-scale purification of viruses for vaccine production.

Our research, which is based on the innovative modification of existing surface display technology, presents a new approach to the process of virus purification. Previous

studies focused on heterologous protein purification and display using surface display technology. First, researchers revealed that subtilisin QK-2 (QK) was successfully separated using GEM surface display technology. In detail, QK-LysM, as a fusion protein, was loaded onto GEM particles by the LysM domain [18]. In other studies, a similar strategy was used to successfully attach various viral antigens, including porcine circovirus type two cap protein [15] and porcine epidemic diarrhea virus N protein [7], to GEM particles. In our research, we focused on PRRSV purification using surface display technology. More specifically, a fusion protein PA-GRFT with two functions, namely GEM binding activity and PRRSV binding activity, was designed. The fusion protein was attached to the surface of GEM particles by means of the PA domain and then PRRSV particles were adsorbed onto the surface of GEM particles by means of the lectin domain, separating them from the cell culture.

Here, it should be noted that the fusion protein PA-GRFT is the key to achieving PRRSV purification. Our data showed that PA-GRFT with sufficient stability was efficiently produced using the *E. coli* system and the resulting expression levels were around 6.6 times higher than those previously reported [6]. PA-GRFT was also proven to bind GEM particles and the PA-GRFT obtained in our study was highly specific to GEM particles. Furthermore, our data showed that 1 U of GEM particles could be saturated with 120 µg of fusion protein via the PA domain, which is in close agreement with previous reports [2]. In addition, the binding between the fusion protein and GEM particles was complete within a short time period, effectively shortening the duration of the purification process. We also assessed the ability of PA-GRFT to bind glycans using a method from a previous study in which researchers evaluated the glycan-binding activity of another lectin wheat germ agglutinin using OVA [29]. Our research confirmed that PA-GRFT could bind to glycans with high affinity and specificity, indicating that PA-GRFT most probably bound to the glycans tethered to the viral envelope glycoproteins.

It has been established that GRFT can bind to a variety of enveloped viruses through their surface glycoproteins [9, 16, 21, 23, 26]. PRRSV is, therefore, a candidate for GRFT binding as it is an example of an enveloped virus that expresses surface glycoproteins. GP5 present on the viral envelope contains two or three putative *N*-glycosylation sites and can, therefore, be heavily glycosylated. Moreover, other glycoproteins including GP2a, GP3 and GP4 are incorporated as multimeric complexes in the viral envelope. Taken together, these facts suggest that the viral glycans are broadly distributed across the whole virion surface, reaching outwards like antennae [14]. Therefore, GRFT would be expected to bind to PRRSV, which provided the basis for the purification process employed in our study.

Given the proposed specific interaction between PRRSV and GRFT, PA-GRFT was used to capture PRRSV by employing a GEM-surface display platform. The purification assay showed that the PRRS virions could be successfully enriched and displayed on GEM particles. Our purification method was highly effective and removed the vast majority of impurities including the serum albumin precursor, which was reported to be the main impurity in a previous study [19]. A variety of methods of virus purification for PRRSV have been reported previously. The traditional method involves density gradient ultracentrifugation [20, 32]; however, this method is difficult to scale-up and cannot meet the demand for vaccine production. Ultrafiltration and chromatography techniques were also investigated for the purification of PRRSV [1, 8]. Hu and colleagues [8] reported that heparin affinity chromatography could be used to capture PRRSV. Although chromatography combined with ultrafiltration removed most cellular and media-related proteins, the cost associated with this method restricted its practical application. In the current study, we report that purified PRRSV can be obtained by a one-step centrifugation procedure without the need for any additional purification steps. Thus our method was easy to scale-up, enabling high throughput, and provided high purity and an appropriate recovery rate. By contrast with the currently available methods, PRRSV purification by GEM surface display technology also had the advantage of low cost. Our approach for the separation of PRRSV from cell culture onto GEM particles may, therefore, be a promising strategy for application in the large-scale purification of viruses for vaccine production.

Interestingly, the GRFT lectin exhibits a broad spectrum of activity [16] against several human enveloped viruses including human immunodeficiency virus and severe acute respiratory syndrome corona virus, which infers that GRFT could also bind to other enveloped animal viruses in addition to PRRSV, such as porcine epidemic diarrhea virus and classical swine fever virus. Thus the purification approach developed here may be applicable more widely to other enveloped animal viruses.

In conclusion, a one-step centrifugation procedure has been developed to successfully separate PRRSV from cell culture and anchored onto the surface of GEM particles via PA-GRFT. This novel approach is easy to perform, cost-efficient and high-throughput and is, therefore, a promising strategy for the large-scale purification of PRRSV for vaccine production. Further studies applying this methodology to the purification of other enveloped animal viruses may lead to a novel universal method of virus purification.

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Compliance with ethical standards

Conflict of interest There is no conflict of interests.

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