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Research Paper

Cloning, Expression and Functionality Evaluation of Recombinant Monoclonal Antibody Against VP1 Capsid Protein of FMD Virus

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ABSTRACT

Introduction: Foot-and-mouth disease (FMD) is a highly contagious viral disease caused by the FMD virus (FMDV), which belongs to the *Aphthovirus* genus in the Picornaviridae family. FMDV is a highly variable RNA virus, and there is limited cross-protection between serotypes and strains. The disease can have devastating economic, social, and environmental impacts. The FMDV capsid is composed of VP1-VP4 structural proteins, with VP1 being the most abundant protein consisting of 213 amino acids. The G-H loop (residues 141-160) of the VP1 capsid protein is highly variable and serves as the main antigenic region. It contains a conserved triplet of amino acids, Arg-Gly-Asp (RGD), which induces the production of protective antibodies against various FMDV types. Monoclonal antibodies (mAbs) play a crucial role in detecting and serotyping FMDV in pathological samples, as well as evaluating protection post-vaccination against FMD.

Materials & Methods: This study explores the expression and function of an engineered recombinant single-chain variable fragment (scFv-mAb) in *Escherichia coli* BL21 (DE3) Rosetta strain as a cost-effective prokaryotic system with high yield. The *scFv-mAb* gene was inserted into the pET28a (+) expression plasmid. *E. coli* cells were transformed with the plasmid, induced with 0.5 mM IPTG, and incubated at 37 ˚C for 12 hours. The protein was purified using a Ni^{2+}-NTA resin column and analyzed by 12% SDS-PAGE for quality assessment. The efficiency and functionality of the scFv-mAb were confirmed using an indirect sandwich (capture) enzyme-linked immunosorbent assay (ELISA).

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Results: The purified *scFv-mAb* concentration was determined to be approximately 2.00 mg/mL using the Bradford assay under optimal conditions. Each well was coated with 400 ng of scFv-mAb based on checkerboard results and the mean of negative serum at a 1:10 dilution. The indirect sandwich ELISA assay yielded an optical density (OD) signal range of 0.3 to 1.5 at a 450 nm wavelength in different positive control treatments.

Conclusion: The ELISA results showed that the scFv-mAb fragment successfully detected serotype O of FMDV. Further research could confirm the potential of this recombinant antibody for broader commercial applications in the future.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of mammals and causes significant economic losses in susceptible cloven-hoofed animals. The virus belongs to the genus Aphtho virus in the family Picornaviridae. Its genome is a positive single stranded RNA, 8.5 kbp length, with a single open reading frame [1].

There are seven different serotypes, A, O, C, Asia1, and Southern African Territories (SAT) 1, 2, and 3. Infection with one serotype does not confer immunity against another. FMD cannot be differentiated clinically from other vesicular diseases; therefore,its laboratory diagnosis is important [1, 2]. The intact virion included an icosahedral capsid structure containing 60 copies of structural proteins (VP1-VP4) and 7 non-structural proteins. The VP1 capsid protein has the highest copy number among all FMDV proteins and consist of 213 amino acids. The G±H loop (residues 141-160) of VP1 capsid protein is highly variable, yet the main antigenic region contains a highly conserved triplet of amino acids, Arg±Gly±Asp (RGD), across FMDV types causing the production of protective antibodies against FMDV types. Studies show that 40% of secreted antibodies against FMDV are stimulated by this loop [2-4].

In this study, a cross-reactive recombinant mAb against conserved RGD region of the G±H loop was designed as an scFv with a flexible linkers for detection of FMDV serotype O. Our goal was to assess the possibility of recombinant scFv-mAb production using an *Escherichia coli* expression system at a low cost and in a short time [2, 3, 5].

2. Materials and Methods

2.1 Engineering and prediction of physicochemical properties of recombinant scFv-mAb

For engineering of the mAb against the G±H loop region of FMD virus VP1 capsid protein (Arg±Gly±Asp, RGD motif), the PDB ID "1ejo" was retrieved and subjected to the required truncations to achieve the desired scFv. The advantages of this antibody included low folding complexities, a well- annotated sequence and 3D structure, and low post-translational modifications, especially glycosylations [3].

The truncation was performed at ARG 2112 (arginine amino acid) in light chain and Lys 2621 in the heavy chain. The antibody domain were fused together using a poly Gly-Ser linker (GSGGGGS). The physicochemical properties of the final engineered FMD virus mAb were analyzed using the ProtParam tool on the ExPasy web server [6]. Through this web server, various parameters were calculated, including: Molecular weight (MW [kDa]), theoretical isoelectric point (pI), estimated half-life under in vitro and in vivo conditions, stability index, aliphatic index, and grand average of hydrophobicity (GRAVY) [6].

2.2. Bacterial strains and culture media

E. coli DH5α (Novagen Co.) was used as a cloning host for the production and maintenance of the expression vector, and *E. coli* BL21(DE3) Rosetta strain (Novagen Co.) was used as the expression host of eukaryotic proteins containing codons rarely used in *E. coli*. Luria Bertani (LB) was used as bacterial culture media [7].

2.3. Plasmid preparation

The recombinant scFv-mAb antibody (50.306 kD) gene cassette was designed using bioinformatic tools and was inserted between NcoI and BamHI restriction. It was then chemically synthesized by Shine Gene Molecular Bio-

tech Co. (Shanghai, China) into the pET28a(+) expression vector. Chloramphenicol and kanamycin antibiotics employed as selectable markers for the Rosetta strain and the pET28a(+) expression vector, respectively.

2.4. Plasmid cloning and extraction

Competent *E. coli* DH5α strain cells (200 µL) were prepared using the $CaCl_2$ method and transformed with pET28a(+) vector (5 µL) via the Novagen heat -shock transformation method [6]. Subsequently, selection for transformants was accomplished by plating on LB agar plates containing kanamycin (35 µg/mL) and incubation at 37 ˚C for 18-24 hours.

Plasmid DNA was extracted and purified from the transformants using the Favor Prep™ Plasmid DNA Extraction Mini Kit, based on kit instructions [8]. Briefly 3 mL of well-grown transformant culture was centrifuged at 11000×g for 1 minute, and the supernatant was discarded completely. The cell pellet was resuspended in 200 µL of FAPD1 buffer (RNase A added) by pipetting. The lysate was clarified by centrifugation at 18,000×g for 5 minutes. During centrifugation, a FAPD column placed in a collection tube. The resulting supernatant was carefully transferred to an FAPD column placed in a collection tube and centrifuged at 11000×g for 30 seconds. After discarding the flow-through, the column was washed with 400 µL of WP buffer (11000×g for 30 seconds) followed by 700 µL of wash buffer (11,000×g for 30 s). Then, the flow-through was discarded, and column was placed back into the collection tube. Next, 700 µL of wash buffer added to the FAPD column and centrifuged at 11000×g for 30 seconds. This process continued by discarding the flow-through and replacing the column back into the collection tube. The column was then centrifuged at full speed (18000×g) for an additional 3 minutes to dry the FAPD column. Then, the FAPD column was placed into a new 1.5 mL microcentrifuge tube. Following this, added 50 µL ~ 100 µL of Elution buffer or ddH_2O was added to the center of the FAPD column membrane. The column was allowed to stand for 1 minute and then centrifuged at full speed (18000×g) for 1 minute to elute the plasmid DNA. Finally the DNA was stored at -20 ˚C. Also, 5 microliters of the extracted plasmid were run on a 0.8% agarose gel at 85 V for 90 minutes, and its quality was checked [8].

2.5. Cloning and protein expression

E. coli BL21 (DE3) Rosetta strain (Novagen) competent cells (200 µL) were prepared using the $CaCl_2$ method and transformed with 5 µL of pET28a(+) vector using the Novagen heat shock transformation method. Subsequently, selection for transformants accomplished by plating on LB agar plates containing chloramphenicol (70 µg/mL), kanamycin (35 µg/mL), and incubated at 37 ˚C for 18-24 hours [7].

Then, transformants were cultured in LB broth containing chloramphenicol (70 µg/mL) and kanamycin (35 µg/mL) and incubated overnight at 37 ˚C with stirring at 210 rpm. Sub-culturing was done at 1:50 (v/v) ratio in 100 mL of fresh LB broth containing chloramphenicol (70 µg/mL) and kanamycin (35 µg/ml) and incubated under the above mentioned conditions. As soon as optical density (OD) at 600 nm reached ~0.8, 1 mL of culture was withdrawn as an expression negative control. The expression of the target protein was then induced by the addition of isopropyl-β-d-galactopyranoside (IPTG; Sigma-Aldrich, St. Louis, USA) at a final concentration of 0.5, 0.75 and 1.0 mM. The culture was incubated at 30 ˚C, 210 rpm. Expression time-course studies were performed at 0, 4, 8 and 12 hours after induction. Finally, pellets were harvested by centrifugation of each sample at 7000×g, 15 minutes at 20 ˚C and stored at -80 ˚C for further processing [9].

2.6. Protein expression analysis and extraction

Collected pellets were resuspended in protein sample buffer (5X) plus 2-mercapto ethanol (2 ME) according to the Laemmli method [7]. Based on the hypothetical MW of scFv (50.306 kDa), the resolving and stacking sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel concentration were selected as 12% and 4%, respectively. Electrophoresis was done in running buffer (25 mM Tris-base, 192 mM glycine, 1% SDS, pH 8.3; CinnaGen Co., Tehran, Iran) at 90 V for 2–3 hours. The gel was stained with a staining solution of 1% Coomassie blue R-250 (Merck, Darmstadt, Germany) and de-stained using a solution of 7% acetic acid (Merck, Darmstadt, Germany), 5% methanol (Merck, Darmstadt, Germany) and 88% water. A standard molecular marker (CinnaGen Co., Tehran, Iran) was run in parallel with the samples to estimate the molecular weights of the proteins. Moreover, for periplasmic proteins extraction, a sonication method (Sonicator; Hielscher Ultra-sound Technology, Brandenburg, Germany) consisting of 5 times ×1 minute sonication and 1 minute interval on ice was followed. Also, 1.0 mM phenyl methyl sulfonyl fluoride (PMSF), (Merck, Darmstadt, Germany) was added to each sample to inhibit potential proteases in extracted samples [7].

2.7. Western blotting (WB) for confirmations of specificity

The specificity of recombinant scFv-mAb was confirmed by WB method. Briefly, SDS-PAGE was followed as described above without gel staining. The sandwich was arranged in a cathode to anode direction as follows: Support pad, Whatman NO.1 filter paper, SDS-PAGE gel, nitrocellulose membrane, Whatman NO.1 filter paper and support pad. The Blotting was implemented at 20 V for 2–3 hours using transfer buffer (25 mM Tris, 192 mM glycine, 20% (v/v) methanol for 1L double-distilled water, pH 8.3). Then, the nitrocellulose membrane was blocked with 5% (w/v) skim milk powder in PBS-T buffer (PBS with 0.05% tween 20 (v/v) overnight at 4 ˚C. Subsequently, washing was done three times with PBST, and the membrane was then incubated with an anti-poly histidine monoclonal antibody at 1:1000 dilutions for 2 hours, at room temperature. After three additional washes, the membrane was soaked in a horseradish peroxidase (HRP)- conjugated anti-His tag antibody (Sigma-Aldrich) solution. DAB solution (Merck, Darmstadt, Germany) was then added as the enzyme chromogen substrate. After incubation in dark place at room temperature and the appearance of the scFv-mAb band color, the reaction stopped with distilled water [7].

2.8. Protein purification and concentration measurement

The production of recombinant scFv-mAb, in either solubilized or insolubilized form, was analyzed by 12% SDS-PAGE gel electrophoresis (Hercules, USA). Then, Ni^{2+}-NTA affinity chromatography (nickel-nitrilotriacetic acid) resin (QIAGEN, USA) was used for protein purification based on affinity to 6×His-tagg at the C-terminus of scFv-mAb. The purification was performed under native conditions with equilibration, washing (plus 20 mM imidazole) and elution (plus 250 mM imidazole) buffers according to the manufacturer's protocol. Collected samples from various purification runs were analyzed using SDS-PAGE as previously described.

The concentration of purified scFv-mAb measured at 595 nm (25 ˚C) using the colorimetric Bradford assay method in comparison with bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, USA) as a standard (0-20 mg/mL). A standard curve was constructed with a serial dilution from 0.00 to 20.00 mg/mL using Graph Pad online server regression tools [10] via a linear regression calculator and plotted against BSA [7, 10, 11].

2.9. Chequerboards and evaluation of scFv-mAb function by using sandwich (capture) enzyme-linked immunosorbent assay (ELISA)

The diagnostic value analysis of purified recombinant scFv-mAb was the most important part of the present study, as it shows its proper folding and functionality. It is performed by indirect sandwich ELISA (S-ELISA, Capture antibody ELISA), which reflects the affinity, folding, function, and specificity of the scFv-mAb. A 96-well flat bottom polystyrene high -bind microplate (Corning Co., USA) was coated with 400 ng/well of scFv- mAb (as a capture antibody) in coating buffer (1.50 g/L Na_2CO_3, 2.93 g/L Na_2HCO_3 in 1000 mL distilled water, pH 9), and incubated overnight at 4 ˚C. Also, BRS (bovine reference serum against FMDV serotype O [BRS-O]) was used as a standard positive antibody control for the FMD virus (virus neutralization test (VNT) Log10≥1/8, 1:10 dilution/well).

The supernatant was then removed, and the wells were washed three times with PBS-T buffer. Subsequently 150 µL of blocking buffer (PBST plus 2% sodium caseinate) was added for 2 hours and incubated at room temperature. The supernatant was removed, and the wells were washed three times with PBS-T. In the chequerboard assay, different concentrations (µg/well) of r-mAb (1.5, 1, 0.5, 0.25, 0.125, 0.061) were used. Also, 2, 1.5, 1, 0.5 µg/well of ultracentrifuged concentrated viral antigen FMDV serotype O2016 (previously prepared by Razi Vaccine & Serum Research Institute, Department of FMD) mixed with 0.03 µg/well of PEG+NACL were added (data not shown). After 45 minutes of incubation at 37 °C and three washes with PBS-T, 100 µL/well of secondary antibody (BRS with VNT 50%≥Log10 1.8) was added in 1:10 dilution. 4 negative controls (non-vaccinated calf serum, age below 6 month) and 2 positive controls (BRS) were also used. The plate was then incubated at 37 °C for 45 minutes and washed three times with PBS-T. Next, 100 µL/well of goat anti -bovine HRP- conjugated antibody was added at a 1:10000 dilution with PBS-T. After 30 minutes of incubation at 37 °C, reactions were developed by adding 100 µL/well of TMB substrate (3, 3′, 5, 5′- tetramethylbenzidine) (IDvet Co., Grabels, France). Finally, 50 µL/well of 1 M H2So4 (Merck, Darmstadt, Germany) was added to stop the reaction. Absorbance at 450 nm was determined by an ELISA microtitre plate reader (Denly, well Scan Co.). The OD of highest dilution of each sera that was 2.5 times bigger than OD of the negative control serum (Mean of negative ±2 SD) was considered as the end-point titer [7, 12, 13].

Table 1. Calculated parameters for recombinant scFv-mAb by using the ExPASy ProtParam tool

Synthetic Fusion Protein Parameters	Recombinant mAb Against FMD Virus (G±H loop Region of the VP1 Capsid Protein)
Number of amino acids	470
MW of synthetic fusion protein	50.306 kDa
Isoelectric point (pI)	6.91
Overall +R & -R	40 & 39
Instability index and half-life estimation (in *E. coli*)	45.24 and >10 hours
Aliphatic index	63.60
Grand average of hydropathicity (GRAVY)	-0.358

3. Results

3.1. scFv-mAb structure analysis

The final scFv-mAb with 470 amino acid lengths was designed and linked together using a peptide linker (GSGGGGS). These nucleotide sequence of the engineered recombinant anti-FMD virus antibody was as follows:

CCATGGGCaaatacctattgcctacggcagccgctggattgttattactcgcggcccagccggccatgGAAGTTATGCTGGTTGAATCTGGTGGTGGTCTGGTTAAACCGGGTGGTTCTCTGAAACTGTCTTGCACCGCTTCTGGTTTCATCTTCAACCGTTGCGCTATGTCTTGGGTTCGTCAGACCCCGGAAAAACGTCTGGAATGGGTTGCTACCATCTCTTCTGGTGGTACCTACACCTACTACCCGGACTCTGTTAAAGGTCGTTTCACCATCTCTCGTGACAACGCTAAAAACACCCTGTACCTGCAGATGTCTTCTCTGCGTTCTGCTGACACCGCTATGTACTACTGCGTTCGTCGTGAAGACGGTGGTGACGAAGGTTTCGCTTACTGGGGTCAGGGTACTGTTGTTACCGTAAGCGCTGCTAAAACCACCCCGCCGTCTGTTTACCCGCTGGCTCCGGGTTCTGCTGCTGCTGCTGCTTCTATGGTTACCCTGGGTTGCCTGGTTAAAGGTTACTTCCCGGAACCGGTTACCGTTACCTGGAACTCTGGTTCTCTGTCTTCTGGTGTTCACACCTTCCCGGCTGTTCTGCAGTCTGACCTGTACACCCTGTCTTCTTCTGTTACCGTTCCGTCTTCTACCTGGCCGTCTGAAACCGTTACCTGCAACGTTGCTCACCCGGCTTCTTCTACCAAAGTTGACAAAAAAATCGTTCCGCGTGGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGACATCGTTCTGACCCAGTCTCCGGCTTCGCTCGCTGTAAGCCTGGGTCAGCGTGCTACCATCTCTTGCCGTGCTTCTGAATCTGTTGACTCTTCTGGTCACTCTTTCATGCACTGGTACCAGCAGAAACCGGGTCAGCCGCCGAAACTGCTGATCTACCGTGCTTCTAACCTGGAATCGGGTATCCCAGACAGGTTCTCTGGTTCTGGCTCTCGTACCGACTTCACCCTGACCATCGACCCGGTTGAAGCTGACGACGTTGCTACCTACTACTGCCAGCAGTCTAACGAAGTTCCGCTGACCTTCGGTGCTGGTACCAAACTGGACCTGAAACGTGCTGACGCTGCTCCGACCGTTTCTATCTTCCCGCCGTCTTCTGAACAGCTGACCTCTGGTGGTGCTTCTGTTGTTTGCTTCCTGAACAACTTCTACCCGAAAGACATCAACGTTAAATGGAAAATCGACGGTTCTGAACGTCAGAACGGTGTTCTGAACTCTTGGACCGACCAGGACTCTAAAGACTCTACCTACTCTATGTCTTC-

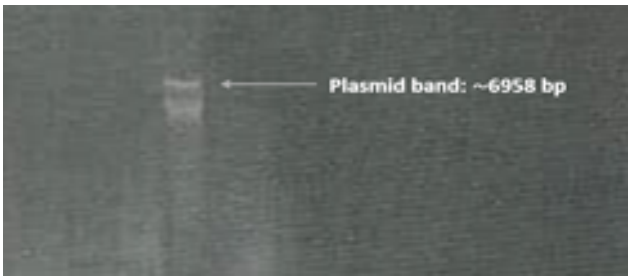

Figure 1. Recombinant plasmid extraction from *E. coli* DH5α was showed in 0.8% (w/v) agarose gel electrophoresis

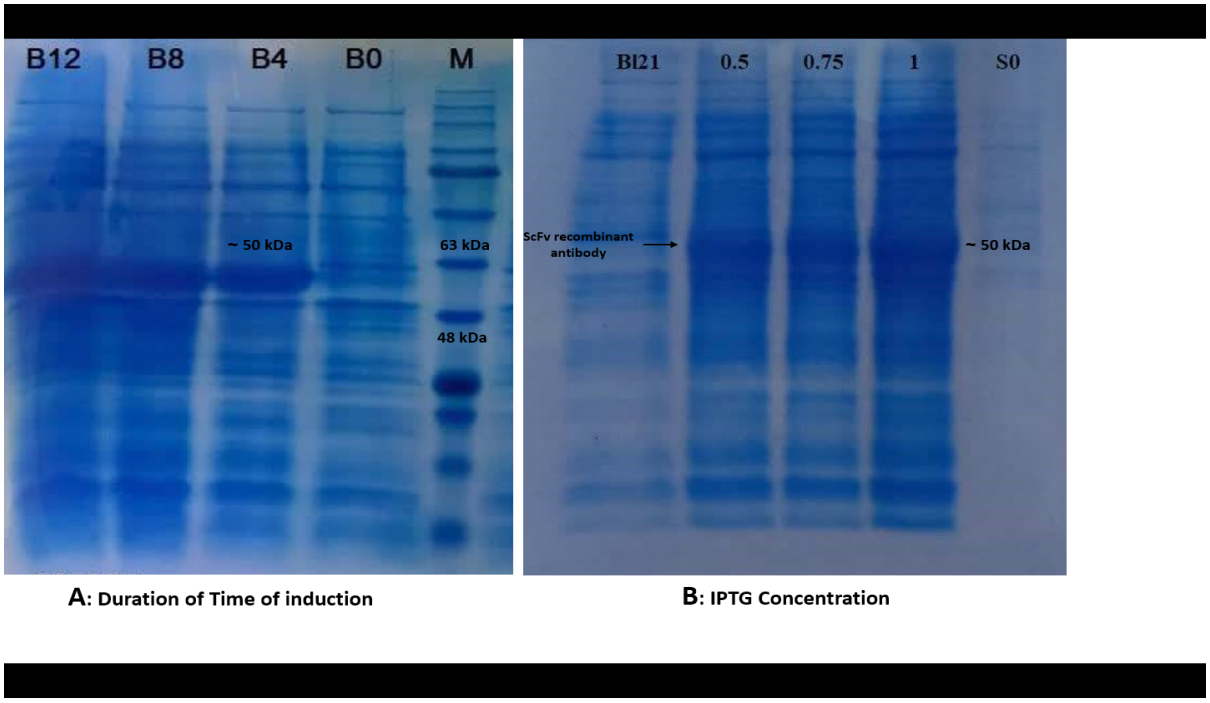

Figure 2. SDS-PAGE of scFv expression results

A) Duration of time at 0, 4, 8 and 12 hours after induction: Lane M: Protein marker (10–250 kDa); B) Expression of total protein in 0.5, 0.75 and 1 mM of IPTG concentration: The arrow shows the corresponding ~50 kDa band of recombinant scFv antibody

TACCCTGACCCTGACCAAAGACGAATAC-GAACGTCACAACTCTTACACCTGCGAAGC-TACCCACAAAACCTCTACCTCTCCGATCGT-TAAATCTTTCAACCGTAACGAATGCGGTCC-GGGTGGTCAGCACCACCACCACCACCAC-CACTGCTAAGGATCC

As shown in the sequence, the signal peptide (PelB signal peptide) at the beginning is showed in lowercase at the 5' terminal, before NcoI restriction enzyme (RE) site (C↓CATGG). A dash is drawn below the start and end codons. At the 3' terminal of the ordered sequence, the His-tag, stop codon, and BamHI RE site (G↓GATCC) have been located.

After initial analysis, the final confirmation of the suitable physicochemical characteristics of the construct's structure was reported (Table 1) [3].

3.2. Plasmid dilution, amplifying and extraction

Plasmid dilution implemented based on manufacturer's instruction with some modifications. The lyophilized vector was dissolved in 100 µL of distilled water to obtain a final concentration of 2 µg/100 µL. From this, 75 µL was stored at -20 ˚C as a stock solution, and 25 µL was used as working solution. Also, pET28a(+) was successfully cloned in *E. coli* DH5α, screened using antibiotic-rich media, and extracted using the FavorPrep™ Plasmid DNA Extraction Mini Kit. At the end of this process, 90 µL was stored at -20 ˚C as extracted plasmid stock, while 10 µL was used as working solution. The concentration of the extracted plasmid solution, measured using a Nanodrop device, was 100 ng/mL. For analysis, 8 µL of the extracted plasmid solution was used for 0.8% (w/v) agarose gel electrophoresis. The results revealed typical, distinct high- purity band corresponding to circular and linear plasmids (~6958 bp) (Figure 1) [14, 15].

3.3. Transformation

The selection of transformants was evidenced by the growth of *E. coli* DH5α on LB agar medium containing (30 µg/mL kanamycin) and *E. coli* BL21(DE3) Rosetta on LB agar medium containing kanamycin (35 µg/mL) and chloramphenicol (70 µg/mL) after 18-24 hours of incubation at 37 ˚C, in compared to the negative control. This confirmed the successful transformation of the pET28a(+) vector into its hosts (data not shown) [16].

3.4. Expression of recombinant scFv-mAb

The transformed *E. coli* BL21(DE3) Rosetta strain, induced with 0.5, 0.75 and 1 mM IPTG and incubated at 37 ˚C for 12 hours, expressed a significant protein fraction in both soluble and sonicated (inclusion body)

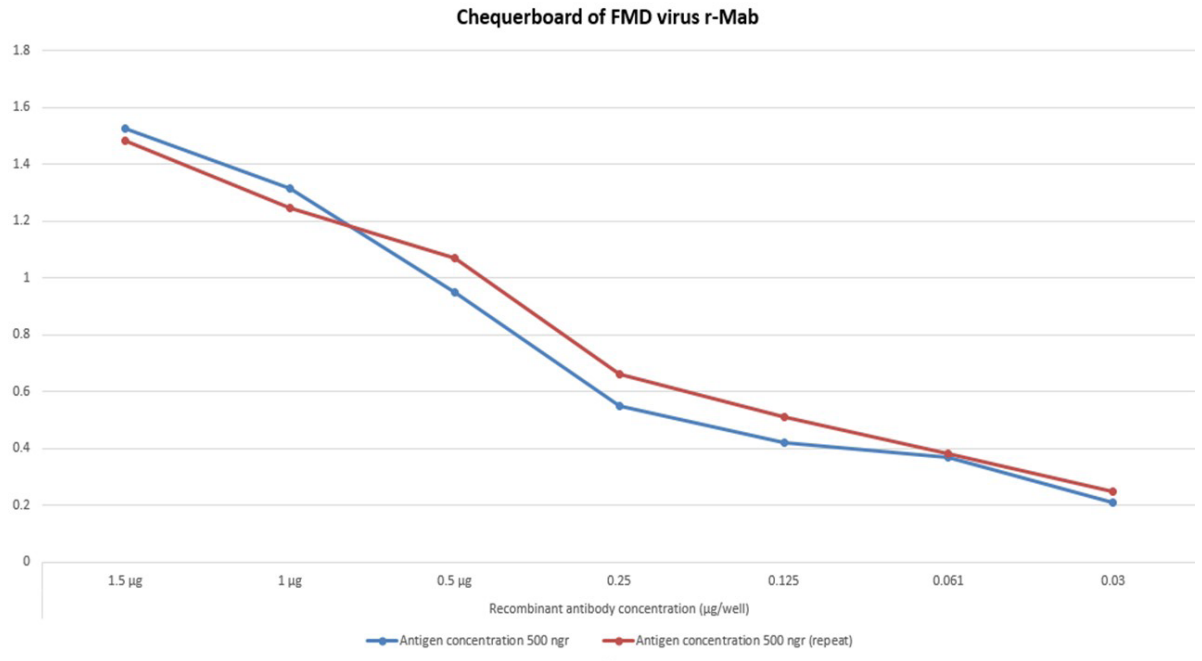

Figure 3. ELISA absorbance results in a chequerboard pattern with different concentrations of recombinant antibody (X axis) and fixed virus antigen 0.5 µg/well (Y axis), 1/10 dilution of secondary BRS antibody and also 1:10000 dilution of Hrp-conjugated tertiary antibody were used. Finally, optimum concentration of 0.4 µg/well of r-mAb antibody was selected for next capture ELISA tests.

(Figures 2A and 2B). All expression samples were run on 12% under reducing condition to confirm the expression of scFv. The SDS-PAGE results clearly showed a distinct band at the predicted position in the induced bacterial cell extract.

As depicted in Figure 2 and evaluated by the presence of ~50 kDa band, the expression was successful for both (0.5 and 1 Mm) IPTG concentrations and duration time of 4 to 12 hours. Additionally, the presence of scFv bands was confirmed by WB [16, 17].

3.5. Concentration calculation of purified scFv-mAb

The optimum conditions for the purification of recombinant scFv-mAb by Ni^{2+}-NTA agarose affinity chromatography included three-step binding process, followed by five washes in the presence of 25 mM imidazole to remove non-specific contaminants. Elution was subsequently implemented using an elution buffer (pH 7.5) containing 0.3 M imidazole to achieve maximum efficiency.

Following a single purification step, SDS-PAGE analysis showed a purity of more than 90%. The concentration of the purified scFv-mAb protein under these optimum condition was calculated to be approximately 2.00 mg/ml using the Bradford assay [14].

3.6. Efficiency evaluation of purified scFv by sandwich (capture) ELISA

By analyzing the chequerboard results, and based on curve break pattern, optical absorption jump, and optical absorption preferences between 0.8 and 1, the optimum concentration for the virus (0.5 µg/well of O2016 serotype) and scFv-mAb (0.4 µg/well) were determined with the highest signal to noise ratio for capture ELISA (Figure 3).

By assessing mean OD of negative serum at a 1:10 dilution against 0.4 µg/well of coated scFv, an OD of 0.3 was eventually selected as the cut-off. ELISA results showed that the scFv-mAb fragment successfully detected FMDV serotype O2016 with signals above 0.3 (optimum concentration of antigen was 0.5 µg/well). Additionally, the mean OD of the 4 negative controls was 0.17. Based on obtained results, when the recombinant scFv-mAb was coated on the plate, it exhibited an OD of $0.3 \leq X \leq 1.5$ at 450 nm wavelength across various positive control treatments.

There was also at least 0.4 OD difference between the purified protein and the crude or lysate protein. Altogether, the present results indicated good sensitivity of

the synthetic recombinant scFv-mAb for the detection of FMDV serotype O whole particles [16, 17].

4. Discussion

This study describes the development of scFv-mAb against the VP1 capsid of FMDV in prokaryotic host for the detection of FMDV serotype O. Although primarily designed for serotype O, it may also be used for the detection of another FMDV serotypes, such as A, C, Asia 1, SAT 1, 2 and 3. Furthermore, it has potential applications in the preparation of antigenic panel (especially in OIE reference countries) and for use in ELISA, diagnostic kits, lateral flow test, virus neutralization test, and as a positive serum control.

Recombinant antibodies have important roles in treatment, research, and diagnosis. While full-length antibodies require mammalian expression systems due to their complex folding and post-translational modification, most antibody fragments and antibody-like molecules are non-glycosylated and can be more conveniently prepared in *E. coli* based expression system. Some commercial recombinant antibodies produced in *E. coli* are currently used in clinical treatment, including Certolizumabpegol (CIMZIA®), Ranibizumab, Brolucizumab, Caplacizumab (trade name Cablivi®) Moxetumabpasudotox (MxP) [18].

In research settings, 1F10 (O UK) has been used as Pan-FMDV mAb [13, 19]. Also, Ochao (2000) reported crystallographic determinations for complexes of 4C4 or SD6 mAb with AnSA peptides, revealing important structural characteristics of the conserved RGD motif [15, 20]. Both studies showed that after binding of the viral peptide to both 4C4 and SD6 mAbs a similar pattern of interactions occurred. It was further established that Asp143 and Leu144 residues were structurally conserved and constituted part of the cell receptor recognition motif [21-23].

In 2000, Ochoa et al. registered the neutralizing monoclonal antibody 4C4 in the PDB database [3].

Its corresponding peptide adopted a compact fold with a nearly cyclic conformation and a specific disposition of the receptor-recognition motif Arg-Gly-Asp (RGD). It was complicated with the Fab fragment of the neutralizing monoclonal antibody 4C4. Although various studies have been conducted on antibody response against FMDV, determination and registration of antibody crystallographic structure remain rare and are limited to this structure.

To date, there are three hundred and twenty-four mAbs introduced for the O, A, C and Asia 1 serotypes of FMDV. Specifically; these include 130 mAbs for serotype O, 108 mAbs for serotype A, 53 mAbs for serotype Asia 1, and 33 mAbs for serotype C [11, 12, 19, 24].

The initial approach involved using an indirect sandwich ELISA with relatively low specificity and sensitivity, employing polyclonal immune sera as both capture (rabbit) and detecting conjugate antibody (guinea pig). Then, it was updated by using polyclonal and mAb as a capture and conjugate antibody, respectively [25, 26].

Grazioli et al. (2020) developed and validated a simplified serotyping ELISA based on monoclonal antibodies for the detection of FMDV serotypes such as O, A, C and Asia 1 [12]. They employed a Pan-FMDV mAb (1F10) as a detector conjugate in a multiplex ELISA, achieving 79% sensitivity compared to the 72% sensitivity of the polyclonal ELISA. This multiplex ELISA is simple, rapid, and stable. So, it could replace existing polyclonal ELISAs for FMDV detection and serotyping. These Pan-FMD antibodies showed valuable results in LFIAs, also a sandwich-type immunoassay combined with a set of well-characterized mAbs. One LFIA antibody work as detecting and identifying O, A and Asia-1 serotypes, while the second antibody enables the detection and differentiation of SAT1 and SAT2 serotypes [2].

Also, another mAb, which is actually a nanobody (M170 Nab), has been produced in *Lama glama* (PDB ID:7DST), a member of the Camelid family. These antibodies are produced specifically against VP3 protein of FMDV serotype O [15].

The production of recombinant antibodies in *E. coli* was first described in 1988 for Fv and scFv fragments. In the present study, we successfully expressed recombinant scFv with relatively high efficiency and performance in the *E. coli* BL21(DE3) Rosetta strain. Numerous studies are in line with our study [16, 27, 28]. The expression of scFv for the detection of different serotypes of FMDV has yielded very effective results. scFv-mAb against 3ABC was effectively produced in *E. coli* in 2014 [17, 29]. In another study in 2003, a known mAb against FMDV serotype O expressed in *E. coli* [30]. Commercial recombinant antibodies produced in prokaryotic and eukaryotic hosts for human and animal diseases include some products from Creative biolabs, Biocompare, Sinobiological and etc.

Further studies could further demonstrate the value of this recombinant antibody in the future, potentially expanding its commercial applications significantly.

It is noteworthy that the cost of scFv production after purification is estimated at approximately 1 USD per 2 mg/mL, which is significantly lower than other expressing platforms such as hybridoma technology and mammalian expression systems.

5. Conclusion

The ELISA assay showed the reliable detection of FMDV serotype O using the recombinant scFv-mAb fragment, indicating its high specificity and sensitivity. Also it support the diagnostic potential of this recombinant antibody and warrant further validation studies to confirm its efficacy and expand its possible commercial applications in both diagnostic and therapeutic contexts for FMDV.

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Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Data availability

The data that support the findings of this study are available upon request from the corresponding author.

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Authors' contributions

Data acquisition, data assessment and elucidation: Mohammad Mahdi Ranjbar; Writing the original draft: Mozhgan Helalinasab; Review and editing: Mohammad Kazem Shahkarami.

Conflict of interest

The authors declared no conflict of interest.

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