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Original Article

Development of a multi-epitope spike glycoprotein vaccine to combat SARS-CoV-2 using the bioinformatics approach

[Desarrollo de una vacuna de glicoproteína spike multiepítopo para combatir el SARS-CoV-2 utilizando el enfoque bioinformático]

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Abstract

Context: The current COVID-19 pandemic has significantly impacted health and socio-economic status worldwide. The only way to combat this situation is to develop an effective vaccine and immunize people around the globe.

Aims: To construct a multi-epitope spike glycoprotein-based vaccine from the SARS-CoV-2 Surabaya isolate using a bioinformatics approach.

Methods: The spike protein was submitted to IEDB, VaxiJen, AllerTOP, and ToxinPred webservers to predict antigenic, non-allergic, non-toxic, B- and T-cell epitopes. To develop a multi-epitope vaccine, an adjuvant cholera toxin B subunit was linked to B-cell and B-cell with T-cell through EAAAK and GPGPG linkers, respectively. The designed vaccine 3D structure development, refinement, and validation were done through PHYRE2, Galaxy Refine, and RAMPAGE webservers. Moreover, the Cluspro-2.0 webserver was used for the molecular docking of the vaccine designed with TLR3**.** The vaccine+TLR3 complex was docked with Surfactant protein A as a control to validate the docking results. Finally, immune-simulation and *in silico* cloning of the vaccine were carried out by C-ImmSim webserver and SnapGene software, respectively.

Results: A multi-epitopic vaccine containing B and T-cell was developed using 392 amino acids with a molecular weight of 40825.59 Da. The docking and immunogenicity results of the vaccine met all established parameters for constructing a quality vaccine. Furthermore, the optimized sequence of the vaccine was successfully cloned in expression vector pET 28 a (+) that yielded a colon of 2724 bp.

Conclusions: The vaccine's immunogenicity demonstrates its effectiveness against SARS-CoV-2 infection. Further confirmatory testing may therefore be performed as soon as possible in the public interest.

Keywords: *in silico*; public health; SARS-CoV-2; spike protein; TLR3-receptor.

Resumen

Contexto: La actual pandemia de COVID-19 ha afectado significativamente la salud y el estado socioeconómico en todo el mundo. La única forma de combatir esta situación es desarrollar una vacuna eficaz e inmunizar a las personas en todo el mundo.

Objetivos: Construir una vacuna basada en glicoproteína de pico de múltiples epítopos a partir del aislado SARS-CoV-2 Surabaya utilizando un enfoque bioinformático.

Métodos: La proteína de pico se envió a los servidores web IEDB, VaxiJen, AllerTOP y ToxinPred para predecir epítopos antigénicos, no alérgicos, no tóxicos, de células B y T. Para desarrollar una vacuna multiepítopo, se unió una subunidad B de la toxina del cólera adyuvante a la célula B y una célula B a una célula T a través de conectores EAAAK y GPGPG, respectivamente. El desarrollo, el refinamiento y la validación de la estructura 3D de la vacuna diseñada se realizaron a través de los servidores web PHYRE2, Galaxy Refine y RAMPAGE. Además, se utilizó el servidor web Cluspro-2.0 para el acoplamiento molecular de la vacuna diseñada con TLR3. El complejo vacuna + TLR3 se acopló con la proteína A del tensioactivo como control para validar los resultados del acoplamiento. Finalmente, la inmunosimulación y la clonación *in silico* de la vacuna se llevaron a cabo mediante el servidor web C-ImmSim y el software SnapGene, respectivamente.

Resultados: Se desarrolló una vacuna multiepitópica que contenía células B y T utilizando 392 aminoácidos con un peso molecular de 40825,59 Da. Los resultados de acoplamiento e inmunogenicidad de la vacuna cumplieron con todos los parámetros establecidos para construir una vacuna de calidad. Además, la secuencia optimizada de la vacuna se clonó con éxito en el vector de expresión pET 28 a (+) que produjo un colon de 2724 pb.

Conclusiones: La inmunogenicidad de la vacuna demuestra su eficacia contra la infección por SARS-CoV-2. Por lo tanto, se pueden realizar más pruebas de confirmación lo antes posible en interés público.

Palabras Clave: *in silico*; proteína de punta; receptor TLR3; salud pública; SARS-CoV-2.

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INTRODUCTION

The SARS-CoV-2 virus causes the current COVID-19 pandemic. It began in Wuhan, China, and spread worldwide, resulting in 4.72 million deaths and unparalleled socio-economic and political consequences (Wu et al., 2020: Zhou et al., 2020; WHO, 2021).

SARS-CoV-2 is a single-stranded RNA virus with a clubbed glycoprotein on its surface (Prajapat et al., 2020; Sarma et al., 2021). In addition, bat and MERS coronaviruses were discovered to have a high level of sequence resemblance (Lu et al., 2020). Thus, many potential vaccine proteins and therapeutic targets exist in SARS-CoV-2. These proteins are primarily responsible for pathogenicity and interaction with the host's body. It is made up of four different types of proteins: envelope protein (E-protein), spike glycoprotein (S-protein), membrane protein (M-protein), and nucleocapsid protein (N-protein), as well as ORFs (open reading frames). The viral transmembrane in these spike glycoproteins has a high bidirectional affinity for the ACE2 receptor on human host cells. It is also regarded as an immune system activator (Srivastava et al., 2019; Ou et al., 2020; Wan et al., 2020).

The surface spike (S) protein of SARS-CoV-2 comprises two subunits, S1 and S2, with S1 being solely responsible for the host cell receptor and S2 housing the membrane fusion machinery. SARS-CoV-2 spike protein is structurally identical to SARS-CoV spike glycoproteins (Weiss and Navas-Martin, 2005). According to previous research, the SARS-CoV-2 spike protein is critical during infection. Spike protein proteolytic stimulation by host cell proteases is thus a crucial determinant (Shokeen et al., 2020). It appears that generating B-cells and T-cells capable of performing immune responses against the SARS-CoV-2 spike protein could be a potential way to treat COVID-19 (Yang et al., 2021).

As a result, we used the SARS-CoV-2 spike glycoproteins as the major of our study to develop our multi-epitope vaccine. Because the S1 subunit of spike protein's receptor-binding domain (RBD) interacts with the ACE2 receptors of host cells, particular antibodies derived from RBD vaccination could significantly suppress viral infiltration by blocking that interaction. As a result, the RBD domains were used to develop vaccines against MERS-CoV and SARS-CoV. (Lan et al., 2020; Zhu et al., 2013).

Immuno-informatics techniques can be used to investigate viral antigens, predict epitopes, and evaluate immunogenicity (Baruah and Bose, 2020; Tahir Ul Qamar et al., 2020a). Furthermore, this strategy could save time and money (Ahmad et al., 2019; Tahir Ul Qamar et al., 2018; 2019). T-cell responses and antibodies can also be used to treat severe respiratory infections (Cockrell et al., 2018). In addition, to prevent SARS-CoV-2 from spreading in households, communities, and healthcare facilities, early diagnosis, isolation, preventing disease, and control measures are essential. Immuno-informatics techniques were used to create treatment approaches against the Ebola virus, MERS-CoV, and Zika virus in many studies (Ahmad et al., 2019; Amer et al., 2018; Ashfaq and Ahmed, 2016; Hui et al., 2018; Tahir Ul Qamar et al., 2019).

This study aimed to identify potential T and B-cell epitopes in SARS-CoV-2 spike proteins from the Surabaya isolate and develop a multi-epitope vaccine. Furthermore, the designed vaccine must be antigenic, non-allergenic, and non-toxic, as well as fulfill all established parameters required for developing a high-quality vaccine, such as physicochemical properties, molecular docking, in-silico cloning, and the ability to trigger immunogenic responses.

MATERIAL AND METHODS

Retrieval of Indonesia whole genome sequence and translation into amino acids

The current study used the whole genome sequence of Surabaya isolate accession ID No. 1366503 retrieved from the GISAID EpiCoV™ Database (Germany). The sequence was subjected to the EXPASY translation tool: https://web.expasy.org/translate/ (Artimo et al., 2012) to translate into amino acids and identify the spike glycoprotein segment within the SAR-CoV-2 sequence. The selected part was confirmed through the NCBI blast protein web: https://blast.ncbi.nlm.nih.gov/Blast.cgi.

B-cell linear epitopes prediction

The identified spike protein was subjected to the Bepipred tool of IEDB webserver: http://tools.iedb.org/bcell/result/ for the prediction 0f B-cell linear epitopes on default threshold (Jespersen et al., 2017).

Identification of T cell epitopes

We employed the IEDB webserver tool: http://tools.iedb.org/mhci/ to predict MHC-1 and MHC-2 bindings in the current study. We used the NeTMH Cpan EL 4.1 method of the IEDB webserver to predict MHC-I epitopes. The chosen amino-acid sequence was submitted to the webserver to evaluate binding affinity with eleven (11) human leukocyte antigen (HLA) supertypes: HLA-A*1, A*2, A*3, A*24,

A*26, HLA-B*7, B*8, B*27, B*39, B*44, B*58 (Dar et al., 2020). Similarly for obtaining MHC-2 bindings we used the IEDB recommended 2.22 and selected Human, HLA-DR (species/Locus) with full HLA_II alleles DRB1*01;01, 03;01, 04;01, 07;01, 07;01, 08;01, 11;01, 13;01, 15;01. Moreover, we selected the length 9-mer for MHC-1 and 15-mer for MHC-2 for humans. In this study, only those peptides with percentile rank ≤1 were considered for the epitope sequence (Dar et al., 2020; Sadat et al., 2021).

Prediction of antigenic, non-allergic, non-toxic for B and T-cell epitopes

The predicted epitopes were subjected to the VaxiJen v2.0 webserver: http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html for antigenicity evaluation (Doytchinova and Flower, 2007), under default parameter of 0.4 thresholds as performed by Oany et al. (2015). The non-allergenicity of epitopes was analyzed by AllerTOP .2 webserver: https://www.ddg-pharmfac.net/AllerTOP/ (Dimitrov et al., 2013). Moreover, the epitopes' non-toxicity was evaluated through the ToxinPred webserver: https://webs.iiitd.edu.in/raghava/toxinpred/algo.php (Gupta et al., 2013).

Physiochemical properties and conservancy analysis

The predicted B-cell epitopes physiochemical analysis based on the grand average of hydropathicity (GRAVY), aliphatic index, theoretical pI, instability index, and molecular weight was done through the protparam tool Expasy: https://web.expasy.org/protparam/ (Abraham Peele et al., 2021). Moreover, the conservancy of the epitopes was illustrated using the conservancy analysis tool present in IEDB Webserver: http://tools.iedb.org/conservancy/ (Naveed et al., 2021).

Population coverage of vaccine

The IEDB-population coverage tool: http://tools.iedb.org/population/ was utilized to evaluate the efficacy of the developed vaccine due to the global nature of the SARS-CoV-2 pandemic (Naveed et al., 2021). Population coverage was assessed with respect to the human leukocyte antigens (HLA-I and HLA-II) binding alleles of epitopes in the specified query section, such as area, country, and ethnicity.

Engineering of the multi-epitopes vaccine

In this study, we used the cholera toxin *B* subunit (NCBI ID= 2CHB_D) as an agent for augmenting the immunogenicity of the construct. The B-cell and T-cell (MHC-1 and MHC-II) were used to engineer multiple epitopes vaccines. Two types of linkers, EAAAK and GPGPG, were used to develop the vaccine. The helex forming EAAAK linker was used for connecting the adjuvant with B-cell epitopes. The linker GPGPG linked the B-cell linear epitopes to the T-cell (MHC-I and MHC_II) epitopes (Dar et al., 2020). Antigenic epitopes with antigenic scores ≥0.5 were utilized to develop the current vaccine. The overlapping of these epitopes was removed finely to shrink the size of the vaccine (Naveed et al., 2021).

Evaluation of physicochemical and solubility characteristics of construct

The constructed vaccine was subjected to the ProtParam webserver of EXPASY: https://web.expasy.org/protparam/ to calculate the physicochemical properties such as GRAVY, instability index, theoretical pI, aliphatic index, and molecular weight (Gasteiger et al., 2005). Furthermore, the solubility of the constructed vaccine was carried out by using the SOLpro tool present in the scratch protein predictor webserver: http://scratch.proteomics.ics.uci.edu/ (Ikai, 1980).

Prediction of the secondary and tertiary structure of vaccine

The SOPMA tool: https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl was applied to obtain the secondary structure of the constructed subunit vaccine (Geourjon and Deleage 1995). In this investigation, the output width was set to 70. The window width, similarity threshold, and several states were set to 17, 8, and 4, respectively. Furthermore, our designed vaccine's tertiary structure (3D) modeling was performed through the PHYRE2 Protein Fold Recognition Server: http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index (Kelley et al., 2015).

Refinement and validation of structure

The obtained 3D model was refined using the Galaxy Refine web server: http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE. The validation of the refined model was carried out by applying the RAMPAGE webserver: http://mordred.bioc.cam.ac.uk/rapper/rampage.php (Naveed et al., 2021).

Molecular docking of designed vaccine

The Cluster-2.0's webserver: https://cluspro.org/home.php was utilized to perform protein-protein docking of the designed vaccine with toll-like receptor 3 (TLR-3) to evaluate their in-

teractions (Kathwate, 2020). Furthermore, we employed surfactant protein A (PDB = 1R13; carbohydrate recognition and neck domains) in the docking procedure as a control (C4) to evaluate our docking results (Safavi et al., 2020).

Vaccine optimization and *in silico* cloning

The J Cat (java codon expression tool) web tool: http://www.jcat.de/ was used to enhance the expression of our protein construct. To accomplish this, we used *E. coli* strain K-12 and the restriction enzyme EcoR1. Then, utilizing SnapeGene software, we employed a pET28 (+) expression vector to colonize this authorized and optimized vaccine sequence (Naveed et al., 2021; Singh et al., 2020).

Immune-simulation of the engineered vaccine

The C-ImmSim webserver: https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php? This server has the potential to represent immune simulations in a manner that is like the original immune repose against any antigen. In this immune-stimulation process, we chose simulation steps 1050 (Kamens, 2015) and three doses of our subunit vaccine on days 1, 28, and 56, respectively.

RESULTS

Retrieval of whole-genome sequence and translation into amino acids

In the current study, the Surabaya isolate SARS-CoV-2 of the RCVTD-ITD, with accession No. 1366503, was downloaded from the GISAID-EpiCoV database on 27 July 2021 for the construction of multiple epitopes vaccine. The whole-genome sequence (WGS) into protein using the Extasy tool. The spike glycoprotein segment was selected from the translated amino acid (protein) sequence. Confirmatory identification was performed through the protein-BLAST of the NCBI database. The selected segments of spike protein consisted of 1451 amino acids (start position = 7321).

Prediction of B-cell epitopes

The identified spike protein sequence was fed into the IEDB webserver's Bepipred tool, which predicted a total of 32 peptides. Based on antigenicity, non-allergenic, and non-toxicity, only two peptides were selected as potential B-cell epitopes to develop a subunit epitope vaccine (Table 1). Moreover, the physicochemical properties of each peptide were obtained through the ProtParam web tool and found the instability index, aliphatic index, grand average of hydropathicity (GRAVY) theoretical pI, and molecular weight of these predicted epitopes (Table 1). The IEDB epitope conservancy tool was used to execute a conservancy analysis on the predicted epitopes, and these epitopes were confirmed to be 100% conserved. The highest score for residues of predicted B-cell epitopes on "Chou and Fasman Beta Turn Prediction" was found to be 0.698 while the minimum to be 0.188 (As the score increases for the residues, the probability to be part of epitopes become higher).

T-Cell prediction and selection

Prediction of MHC-I epitopes

The selected spike protein was employed in the IEDB web server tool for MHC-I epitopes and selected HLA supertypes: HLA-A*1, 2, 3, 24, 26, HLA-B*7, 8, 27, 39, 44, 58. A total of 36 epitopes of 9-mer length were considered based on percentile rank ≤1. We evaluated these identified MHC-1 epitopes for antigenicity and found 11 epitopes (Table 2). These antigenic 11 epitopes were further analyzed based on antigenicity score ≥0.5, non-allergenicity, and non-toxicity, and only 8 MHC-I binding epitopes were obtained (Table 3). The maximum antigenic score was depicted as 1.5122 for selected epitopes.

Prediction of MHC-II epitopes

The spike protein MHC-II binding epitopes prediction was also made in the IEDB web server tool for MHC-II epitopes. For this we selected 15-mer length and HLA-DR, HLA_II alleles DRB1*01;01, 03;01, 04;01, 07;01, 07;01, 08;01, 11;01, 13;01, 15;01. A total of 57 MHC-II epitopes were selected under the set criteria of percentile rank ≤1. These epitopes were analyzed for non-toxicity, antigenicity, and non-allergenicity and obtained 34 potential epitopes. Furthermore, these selected epitopes were evaluated on antigenicity score ≥0.5 and found only 7 epitopes (Table 4).

Engineering of the multi-epitopic vaccine

This study used 2B-cell, 8MHC-1, and 7 MHC-II epitopes with antigenic scores ≥0.5 to engineer a multi-epitopes vaccine. We used the cholera toxin *B* subunit (NCBI Id = 2CHB_D) as an agent to boost the immunogenicity of the construct in this study. EAAAK linker was used to link adjuvant with B-cell epitopes, and GPGPG linker was applied to connect the B-cell with MHC-I, and MHC-II epitopes, respectively. In addition, the overlapping of these epitopes was removed to reduce the length and size of the vaccine. Finally, a vaccine construct was engineered based on subunit multiple epitopes with a molecular weight of 40825.59 Da and 392 amino acids.

Table 1. Predicted B-cell epitopes selected for vaccine construction.

No.	Peptides	Antigenic	Allergen	Toxic	Molecular weight	Theoretical pI	Instability Index	Aliphatic Index	GRAVY
1	SQPFLMDLEGKQGNFKNL	Yes	Non	Non	2066.36	5.79	28.57	65.00	-0.728
2	VNNSYECDIP	Yes	Non	Non	1153.23	3.67	61.78	68.00	-0.650

Table 2. High affinity MHC-I binding epitopes of spike protein in Surabaya isolates.

No.	Allele	Peptide	Score	Percentile rank	Antigen
1	HLA-B*27:02	SRFPNITNL	0.96802	0.01	**0.8964**
2	HLA-B*07:02	SPRRARSVA	0.918799	0.04	**0.7729**
3	HLA-B*39:01	SRFPNITNL	0.900191	0.03	**0.8964**
4	HLA-A*24:02	VYDPLQPEL	0.851829	0.04	**0.4525**
5	HLA-A*02:06	VVFLHVTYV	0.850041	0.06	**1.5122**
6	HLA-A*02:01	LLFNKVTLA	0.803506	0.08	**0.6150**
7	HLA-A*02:06	FTISVTTEI	0.796069	0.08	**0.8535**
8	HLA-B*44:03	YEQYIKWPW	0.781402	0.09	**0.8690**
9	HLA-B*44:03	ADAGFIKQY	0.769592	0.1	**0.4271**
10	HLA-B*44:03	AEIRASANL	0.764604	0.1	**0.7082**
11	HLA-B*39:01	VRDPQTLEI	0.755419	0.06	**0.7025**

Table 3. High affinity MHC-I binding antigenic, non-allergic, non-toxic epitopes of spike protein in Surabaya isolates.

No.	Peptide	Score	Percentile rank	Antigenic	Allergic	Toxic
1	SRFPNITNL	0.96802	0.01	Yes	Non	Non
2	SPRRARSVA	0.918799	0.04	Yes	Non	Non
3	SRFPNITNL	0.900191	0.03	Yes	Non	Non
4	VVFLHVTYV	0.850041	0.06	Yes	Non	Non
5	LLFNKVTLA	0.803506	0.08	Yes	Non	Non
6	FTISVTTEI	0.796069	0.08	Yes	Non	Non
7	AEIRASANL	0.764604	0.1	Yes	Non	Non
8	VRDPQTLEI	0.755419	0.06	Yes	Non	Non

Table 4. High affinity MHC-II antigenic, non-allergic, non-toxic epitopes of spike protein in Surabaya isolates.

No.	Peptide	Percentile rank	Antigenic	Allergic	Toxic
1	VVLSFELLHAPATVC	0.03	Yes	Non	Non
2	DQLTPTWRVYSTGSN	0.15	Yes	Non	Non
3	RVVVLSFELLHAPAT	0.24	Yes	Non	Non
4	AIPTNFTISVTTEIL	0.40	Yes	Non	Non
5	IPTNFTISVTTEILP	0.52	Yes	Non	Non
6	TPINLVRDLPQGFSA	0.59	Yes	Non	Non
7	RFQTLLALHRSYLTP	0.84	Yes	Non	Non

Table 5. Population coverage of designed vaccine.

Population/area	Class I			Class II			Class combined		
	Coverage	Average hit	pc90	Coverage	Average hit	pc90	Coverage	Average hit	pc90
Europe	64.81%	0.86	0.28	69.9%	1.24	0.33	89.41%	2.1	0.94
North America	57.52%	0.74	0.24	67.59%	1.15	0.31	86.23%	1.89	0.73
North Africa	36.88%	0.43	0.16	55.03%	0.9	0.22	71.61%	1.33	0.35
South Asia	23.71%	0.34	0.13	61.7%	1.1	0.26	70.78%	1.44	0.34
East Asia	53.33%	0.88	0.21	33.77%	0.54	0.15	69.09%	1.42	0.32
West Indies	49.71%	0.6	0.2	39.95%	0.71	0.17	69.8%	1.31	0.33

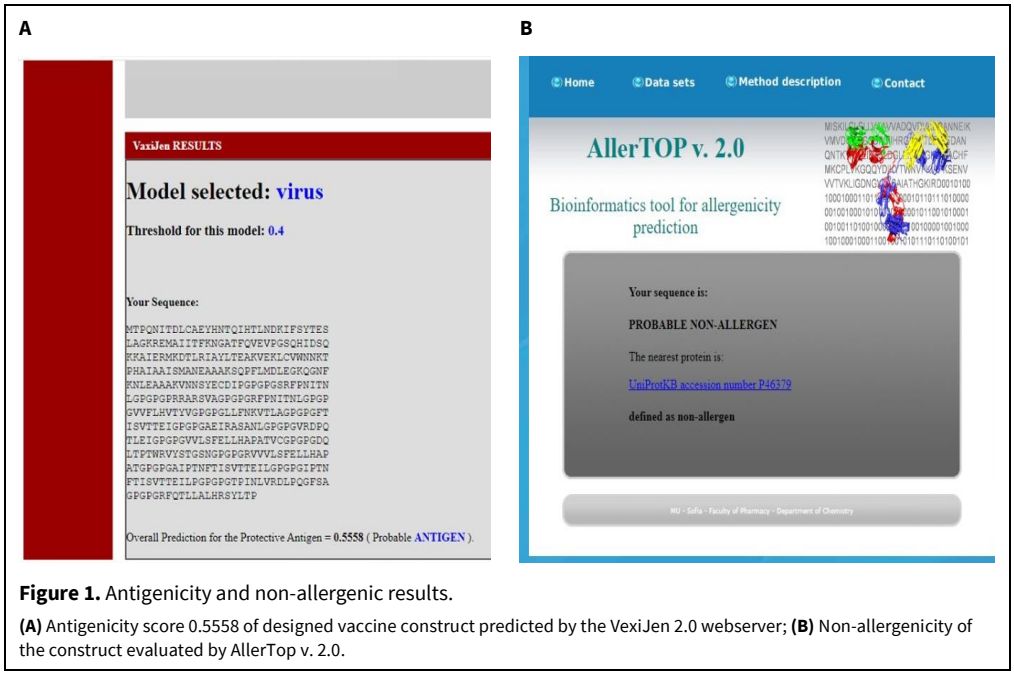

Figure 1. Antigenicity and non-allergenic results.

(A) Antigenicity score 0.5558 of designed vaccine construct predicted by the VexiJen 2.0 webserver; **(B)** Non-allergenicity of the construct evaluated by AllerTop v. 2.0.

Constructed subunit vaccine sequence

MTPQNITDLCAEYHNTQIHTLNDKIFSYTESLA GKREMAIITFKNGATFQVEVPGSQHIDSQKKAIER MKDTLRIAYLTEAKVEKLCVWNNKTPHAIAAISM ANEAAAKSQPFLMDLEGKQGNFKNLEAAAKVNN SYECDIPGPGPGSRFPNITNLGPGPGPRRARSVAGP GPGRFPNITNLGPGPGVVFLHVTYVGPGPGLLFNK VTLAGPGPGFTISVTTEIGPGPGAEIRASANLGPGP GVRDPQTLEIGPGPGVVLSFELLHAPATVCGPGPG DQLTPTWRVYSTGSNGPGPGRVVVLSFELLHAPAT GPGPGAIPTNFTISVTTEILGPGPGIPTNFTISVTTEIL PGPGPGTPINLVRDLPQGFSAGPGPGRFQTLLALH RSYLTP.

Antigenic, non-allergic, and non-toxicity analysis of designed vaccine

The constructed subunit multiple epitopes-based vaccines were tested using the VaxiJen- 2.0 website. It was found to be antigenic with a score of 0.5558**.** The designed construct was then tested for antigenicity without adjuvant and received a score of 0.5527**,** indicating that it is also antigenic. These findings suggested that the construct would be antigenic in both situations, whether adjuvant-linked or not. Furthermore, the allergenicity status of this vaccine was confirmed to be non-allergic utilizing the Aller-Top V.2.0 webserver, whether adjuvant linked or not (Fig. 1).

Population coverage

The selected epitopes of our construct depicted 89.41, 86.23, 71.61, 70.78, and 69.09% of Europe, North America, South Africa, South Asia, and East Asia, respectively (Table 5). These findings show that the construct could be used to combat SARS-CoV-2 worldwide.

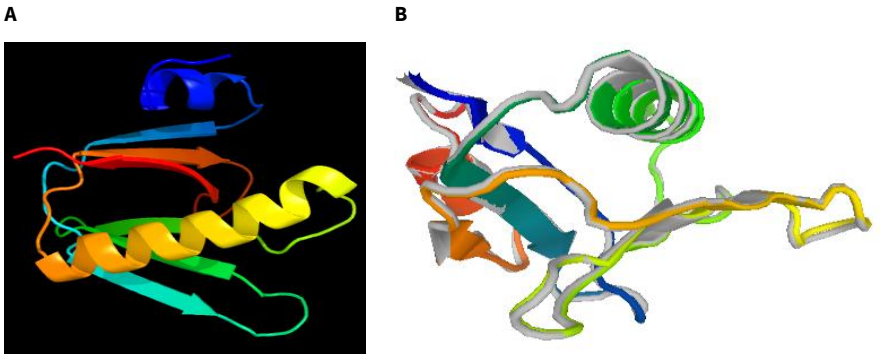

Figure 2. Tertiary structures.

(A) 3D structure developed by PHYRE-2 webserver; **(B)** Refined 3D, structure developed through Galaxy Refine webserver.

Evaluation of physicochemical and solubility characteristics of the designed vaccine

The physiochemical characteristics of the designed vaccine were calculated using the ProtParam web server. The molecular weight of the designed protein construct was 40825.59 Da, with a pI of 8.30 indicating that the protein is essential. The II (instability index) was found to be 30.74, marking its stability, as any >40 suggests that the protein is unstable (Jespersen et al., 2017). The aliphatic index was found to be 80.38, indicating a highly thermostable protein (Ikai, 1980). Furthermore, the GRAVY was found to be -0.146, showing the hydrophobicity of the protein. This negative value (-0.146) of GRAVY confirmed that our construct is hydrophilic (Ali et al., 2017; Chang and Yang, 2013). The SOLpro online server was used to determine the solubility rate of the designed subunit vaccine construct, which was 0.953680.

Secondary structure prediction of vaccine

The SOPMA tool was used to estimate the secondary structure of the built subunit vaccine and discovered that the design was composed of Alpha helix 22.70 %, Extended strand 27.55 %, Beta turn 4.08 %, and Random coil 45.66 %.

Tertiary structure development, refinement, and validation

We used PHYRE2 webservers for the development of the 3D structure. Initially, the PHYRE2 webserver predicted 120 models, but we chose model-1 based on 100% confidence and maximum coverage (Fig. 2). The Galaxy refines webserver refined this predicted 3D model, which yielded 5 different refined models. We selected Galaxy server refined model-3 (Fig. 2), for the further protein-protein docking process based on different parameters best-predicted scores: MolProbit score= 1.402, GDT-HA score= 0.9830, Clash score score= 6.6, RMSD score= 0.324, Rama favored score= 99.0, and poor rotamers score=1.1. The refined model was validated through the RAMPAGE webserver that predicted the good quality of our refined model. The selected model's validation showed: 96.8 % of the residues in favored regions, 3.2 % in additional allowed regions, 0.0% in disallowed regions, and 0.0% in generously allowed regions (Fig. 3). The only 3D model with more than 70% of its residues in the favored region is deemed a quality structure (Chukwudozie et al., 2020).

Molecular docking (MD) of engineered vaccine with TLR3 receptor

The interaction of an engineered vaccine and TLR3 has been investigated through protein-protein docking of the Cluspro.2.0 webserver. The Cluspro server displayed a total of 29 different models. We evaluated these projected models and picked model-1 because it had the lowest energy score of interaction -728.2 Kcal/mol with the highest clustering members 73 (Fig. 3). In addition, the docked complex was visualized by using the PyMol software as well. Furthermore, the energy of the vaccine+TLR3+ C4 complex (-688.9 Kcal/mol) was higher than the energy of the vaccine + TLR-3, showing that our vaccine has a more robust interaction than the control model (Fig. 3).

Optimization and *in silico* cloning of vaccine

We utilized the J-Cat webserver to maximize the expression of our protein construct. We obtained an optimized sequence of 1176 nucleotides. Furthermore, we computed its CAI-value of 0.89 and the GC Content value of 57.23. These findings showed our engineered vaccine's ability to express in *E. coli* as a host cell. We chose the restricted sites HpaI as the starting point and SmaI as the ending cut point for colonizing the optimized sequence into expression vector pET28(+). We found a final cloned vector of 2724 bp using the SnapeGene software (Fig. 4).

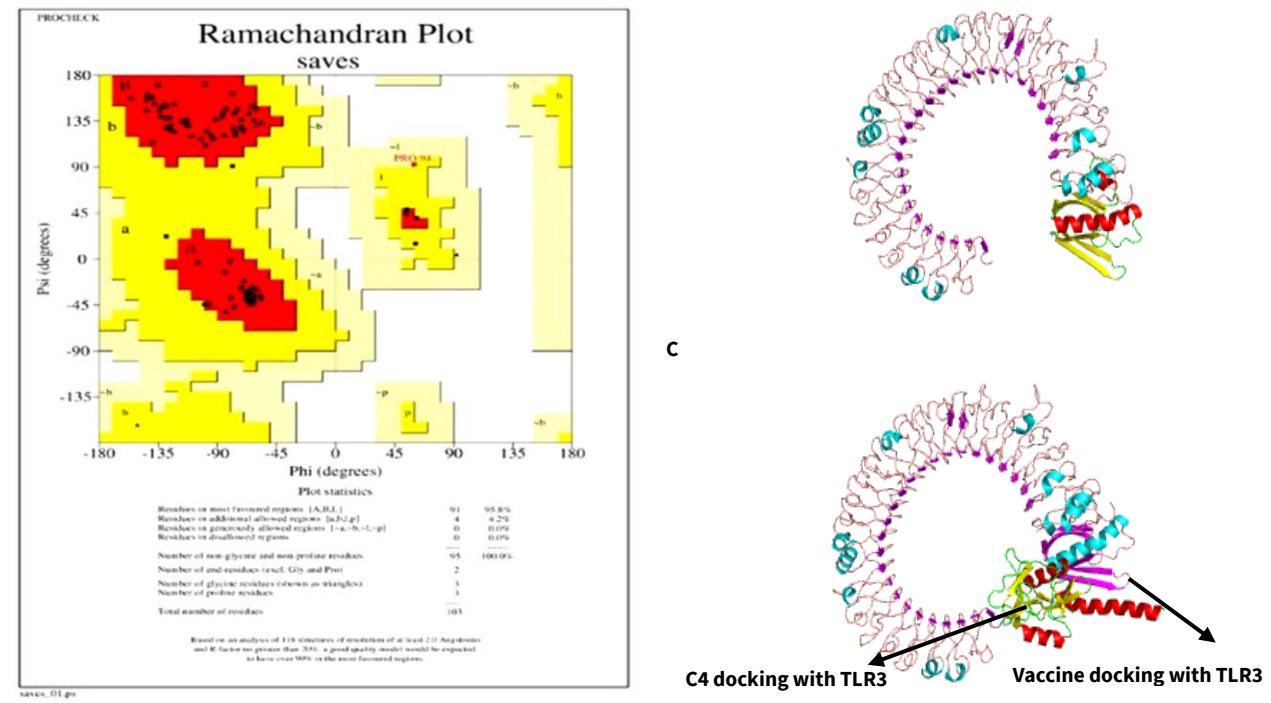

Figure 3. Validation and molecular docking results.

(A) Vaccine validation design through RAMPAGE web server depicting 95.8 % of the residues to be in favorite regions score; **(B)** Vaccine construct and TLR 3 receptor docking interaction; **(C)** Docking of vaccine + TLR3 complex with control C4.

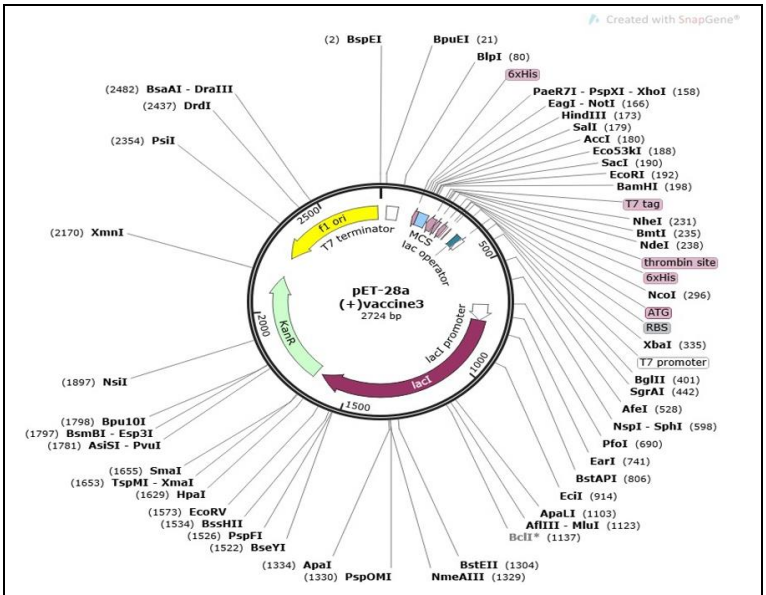

Figure 4. *In silico* cloning of optimized sequence construct into the pET-28 a (+) yielded a colon of 2724bp using the Snape-gene software.

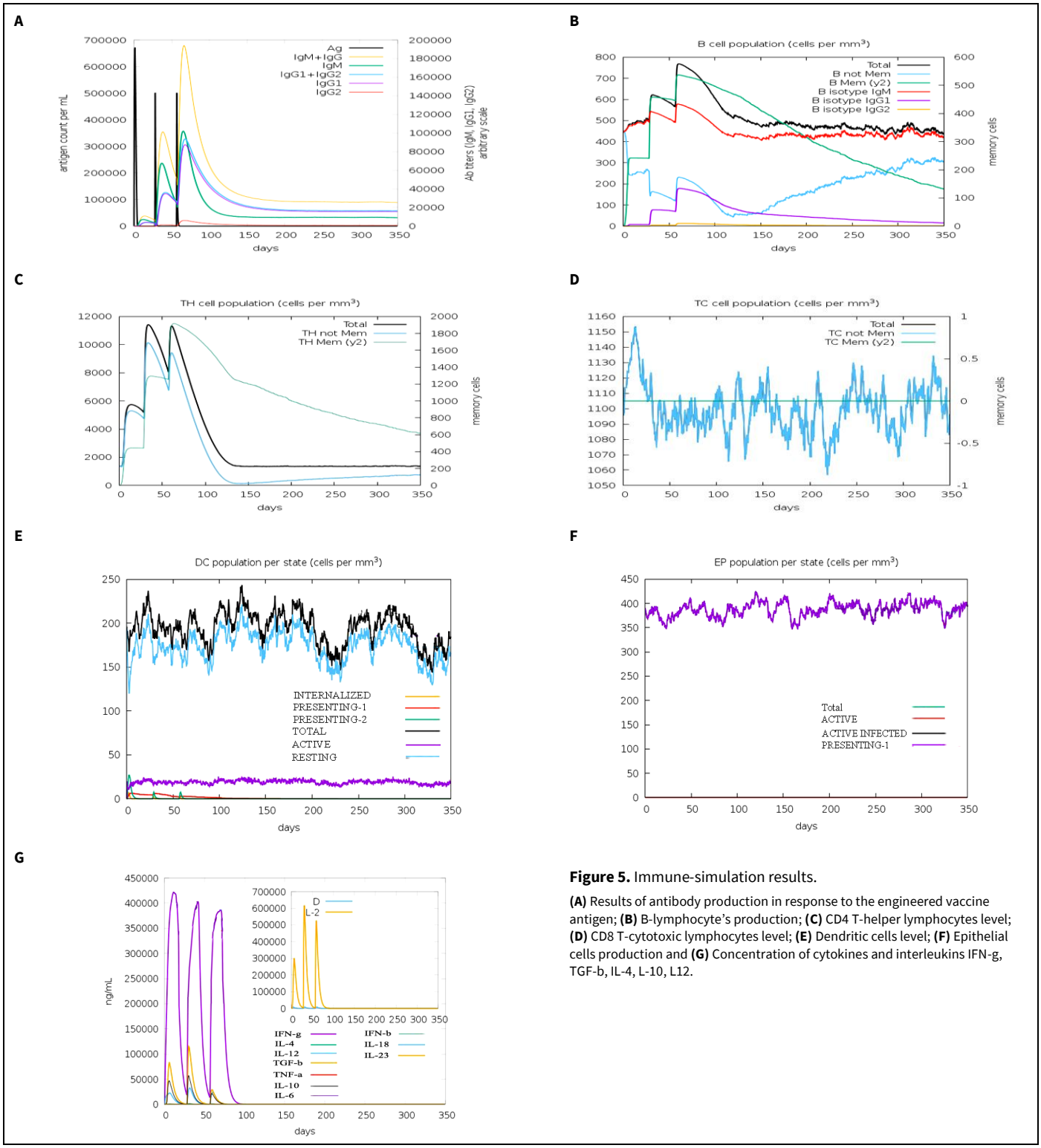

Figure 5. Immune-simulation results.

(A) Results of antibody production in response to the engineered vaccine antigen; **(B)** B-lymphocyte's production; **(C)** CD4 T-helper lymphocytes level; **(D)** CD8 T-cytotoxic lymphocytes level; **(E)** Dendritic cells level; **(F)** Epithelial cells production and **(G)** Concentration of cytokines and interleukins IFN-g, TGF-b, IL-4, L-10, L12.

Immune simulation of the engineered vaccine

The C-ImmSim webserver was applied to assess the immunological responses of our construct using 1050 simulation steps and an injection time scale based on three doses on days 1, 28, and 56. Our engineered vaccine performed admirably. The primary response was identified as high-level IgM, and secondary and tertiary responses as high levels of IgG1 + IgG2, IgM + IgG, IgG1, IgG2, and B cell population. Furthermore, the antibody (Ab) titer increased as the antigen concentration decreased. It was observed that the Ab titer remained stable after the third injection throughout the year. In this study, CD4 and CD8 displayed enhanced responses comparable to memory cells, indicating T cell immunogenicity, part of our construct. Furthermore, repeated exposure resulted in

a considerable rise in IFN-γ, TGF-β, IL-4, IL-10, and IL-12 (Fig. 5).

DISCUSSION

The most effective counter-strategies against the COVID-19 pandemic are therapeutic interventions and effective vaccination. SARS-CoV-2, a highly contagious disease, is currently the world's most severe human threat. Thus, the time has come for an effective vaccine because only vaccination can eradicate and control this viral infection. We have eradicated and controlled smallpox, polio, and measles from the world through immunization. However, developing a novel coronavirus-19 vaccine is difficult due to a lack of immunogenic information about the virus and information about the virus's required immunogenic responses to counter SARS-CoV-2 (Raoult et al., 2020). Epitope-based vaccines for many viruses, such as the rhinovirus and the dengue virus, have successfully indicated their ability to protect against the targeted virus (Ahmad et al., 2020). However, multiple resistance was observed in the SARS-CoV-2 RNA vaccines due to rapid genomic mutations (Zeng et al., 2021). As a result, scientists are focusing their efforts on developing protein-based vaccines to combat SARS-CoV-2 infection and mutational resistance. Because antibodies to spike proteins have previously been shown to prevent infection (Martínez-Flores et al., 2021), the current study was designed to investigate the immunogenic potential of spike glycoproteins in a Surabaya isolate against COVID-19 through bioinformatics tools. We targeted Surabaya isolates for vaccine development because local viral vaccines are more effective and efficient against viral infections (Indriani, 2001).

The traditional *in vitro* and *in vivo* approaches to identifying antigens, predicting epitopes, allergenicity, and toxicity for vaccine development are time-consuming and expensive. In contrast, bioinformatics techniques have proven to be more effective, easing and speeding up vaccine development (Dong et al., 2020; Olvera et al., 2020; Poran et al., 2020). The current study is novel because it evaluated the immunogenic potentials of spike glycoprotein present in Surabaya isolates under accession ID 1366503 and provided information on a multi-epitopic vaccine engineered using the Spike protein as all other details required for the first stage of vaccine development. The current investigation identified the B-cell and T-cell epitopes responsible for innate and adaptive immunity induction (Singh et al., 2020).

Furthermore, T-cell-derived immunotherapy is thought to be long-lasting (Phan et al., 2017). To create a multi-epitopic spike protein vaccine, we linked the 2B-cell, 8 MHC-1, and 7 MHC-II epitopes with antigenicity scores ≥0.5. This study used cholera toxin *B* subunit as an agent to increase the immunogenicity of the vaccine construct because the affinity of the adjuvant causes an increase in T-cell and B-cell responses via the innate immune system (McKee et al., 2007; McCartney et al., 2009; O'Hagan and De Gregorio, 2009). The engineered vaccine has a molecular weight (MW) of 40.82559 KDa, which is a significantly good candidate vaccine because the recommended MW for a good quality vaccine construct is <110 KDa (Khalil et al., 2018; Reche et al., 2014). The antigenicity of the suggested vaccine construct was determined to be 0.5558, which is comparable to the previously reported 0.5430 (Naveed et al., 2021). It was discovered to be non-allergenic since the allergenicity of the produced vaccine is a serious problem in vaccine production (Oany et al., 2014). Tertiary structure modeling is essential for better understanding protein function at the proteome level. (Houston et al., 2018). The PHYRE2 webserver was used to create the tertiary model of the constructed vaccine, and the chosen model had 100% confidence and maximal coverage. Furthermore, the refinement is required for critically lowering the error 3D structure of construct, particularly the Galaxy web tool, which not only provides a template-based model but also refines the different terminus and loops of the 3D model (Ko et al., 2012), which is required for molecular docking with the receptor. In this study a refined model on best parameters scores (MolProbit score = 1.402, GDT-HA score = 0.9830, Clash score score = 6.6, RMSD score = 0.324, Ramachandra score = 99.0, and poor rotamers score = 1.1.) was selected. The RAMPAGE webserver validated the refined model and found 95.8% residues in favored regions. The refined structure is considered a quality structure with >70% of its residues in the favored region. It is considered a quality structure (Chukwudozie et al., 2020). The protein-protein molecular docking of the vaccine construct and the TLR-3 were successfully carried out and found strong bonding interaction between them. We used the TLR3 human receptor in our study because it is important in eliciting immune responses against virus pathogenicity (Tahir Ul Qamar et al., 2020b). We obtained an optimized 1176 nucleotide sequence with a GC content of 57.22 and a CAI of 0.89 in this study. The GC value in our study is within the recommended range of 30-70, which is considered efficient for translational effectiveness. The CAI value is also within the defined range of 0.8 to 1.0, which is considered effective vaccine construct expression in E. coli (Chukwudozie et al., 2021). For in silico colonization, the vaccine construct was inserted into the expression vector pET28a (+). The vaccine design expression could be evaluated in a bacteriological system. Using the SnapeGene program, a final cloned vector of 2724

bp was discovered. A similar approach was reported by Jyotisha and Qureshi (2020). Our vaccine-elicited both cellular and humoral responses, with the significant activity of IL-23, IL-12, IL-18, IL 4, IL-6, and IL-10, indicating a high level of IFN-y production.

Furthermore, the constructed vaccine produced higher IgG1, IgG2, IgM, IgM + IgG, and IgG1 + IgG2. Furthermore, it was shown that IgM and IgG titers increased immediately after the third vaccine. Simultaneously, it was observed that as antigen levels decreased, immunoglobulin levels increased. These outcomes confirm the effectiveness of the developed vaccine (Arumugam and Varamballi, 2021). The current spike protein-based multi-epitope subunit vaccine development showed promising results when analyzed through viroinformatics tools. However, the actual efficacy of the product will remain unknown without lab and field testing.

CONCLUSION

Using bioinformatic approaches, we can reduce the time and costs of identifying epitopes in laboratories. In this study, we developed a spike glycoprotein-based SARS-CoV-2 multi-epitope vaccine from a Surabaya, Indonesia, isolate using bioinformatic techniques and found its robust interaction by docking with the TLR-3 receptor. The immune-simulation analysis revealed that the developed vaccine elicits both humoral and cellular immune responses. In addition, *in silico* colonization of the vaccine in an expression vector demonstrated its ability to be produced on a large scale. The study's findings suggested that it should be tested further in the laboratory and field for public health interests.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Abraham Peele K, Srihansa T, Krupanidhi S, Ayyagari VS, Venkateswarulu TC (2021) Design of multi-epitope vaccine candidate against SARS-CoV-2: A in-silico study. J Biomol Struct Dyn 39(10): 3793–3801.

Ahmad B, Ashfaq UA, Rahman MU, Masoud MS, Yousaf MZ (2019) Conserved B and T cell epitopes prediction of Ebola virus glycoprotein for vaccine development: An immuno-informatics approach. Microb Pathog 132: 243–253.

Ahmad I, Ali SS, Zafar B, Hashmi HF, Shah I, Khan S, Suleman M, Khan M, Ullah S, Ali S, Khan J (2020) Development of multi-epitope subunit vaccine for protection against the norovirus' infections based on computational vaccinology. J Biomol Struct Dyn 9: 1–12.

Ali M, Pandey RK, Khatoon N, Narula A, Mishra A, Prajapati VK (2017) Exploring dengue genome to construct a multi-epitope-based subunit vaccine by utilizing immunoinformatics approach to the battle against dengue infection. Sci Rep 7(1): 9232.

Amer H, Alqahtani AS, Alaklobi F, Altayeb J, Memish ZA (2018) Healthcare worker exposure to Middle East respiratory syndrome coronavirus (MERS-CoV): Revision of screening strategies urgently needed. Int J Infect Dis 71: 113–116.

Artimo P, Jonnalagedda M, Arnold K, Baratin D, Csardi G, De Castro E, Duvaud S, Flegel V, Fortier A, Gasteiger E, Grosdidier A (2012) ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res 40(W1): W597–W603.

Arumugam S, Varamballi P (2021) In-silico design of envelope-based multi-epitope vaccine candidate against Kyasanur forest disease virus. Sci Rep 11(1): 17118.

Ashfaq UA, Ahmed B (2016) De novo structural modeling and conserved epitopes prediction of Zika virus envelop protein for vaccine development. Viral Immunol 29(7): 436–443.

Baruah V, Bose S (2020) Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. J Med Virol 92(5): 495–500.

Chang KY, Yang JR (2013) Analysis and prediction of highly effective antiviral peptides based on random forests. PloS One 8(8): e70166.

Chukwudozie OS, Chukwuanukwu RC, Iroanya OO, Eze DM, Duru VC, Dele-Alimi TO, Kehinde BD, Bankole TT, Obi PC, Okinedo EU (2020) Attenuated subcomponent vaccine design targeting the SARS-CoV-2 nucleocapsid phosphoprotein RNA binding domain: *In silico* analysis. J Immunol Res 2020: 2837670.

Chukwudozie OS, Gray CM, Fagbayi TA, Chukwuanukwu RC, Oyebanji VO, Bankole TT, Adewole RA, Daniel EM (2021) Immuno-informatics design of a multimeric epitope peptide-based vaccine targeting SARS-CoV-2 spike glycoprotein. Plos One 16(3): e0248061.

Cockrell AS, Johnson JC, Moore IN, Liu DX, Bock KW, Douglas MG, Graham RL, Solomon J, Torzewski L, Bartos C, Hart R (2018) A spike-modified Middle East respiratory syndrome coronavirus (MERS-CoV) infectious clone elicits mild respiratory disease in infected rhesus macaques. Sci Rep 8(1): 10727.

Dar HA, Waheed Y, Najmi MH, Ismail S, Hetta HF, Ali A, Muhammad K (2020) Multiepitope subunit vaccine

design against COVID-19 based on the spike protein of SARS-CoV-2: An *in silico* analysis. J Immunol Res 2020: 8893483.

Dimitrov I, Flower DR, Doytchinova I (2013) AllerTOP-a server for in silico prediction of allergens. BMC Bioinform 14(suppl. 6): S4.

Dong R, Chu Z, Yu F, Zha Y (2020) Contriving multi-epitope subunit of vaccine for COVID-19: Immunoinformatics approaches. Front Immunol 11:1784.

Doytchinova IA, Flower DR (2007) VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinform 8(1): 4.

Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A (2005) Protein identification and analysis tools on the ExPASy server. The Proteomics Protocols Handbook, pp. 571–607.

Geourjon C, Deleage G (1995) SOPMA: Significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Bioinform 11(6): 681–684.

Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Open-Source Drug Discovery Consortium, Raghava GP (2013) *In silico* approach for predicting toxicity of peptides and proteins. PloS One 8(9): e73957.

Houston S, Lithgow KV, Osbak KK, Kenyon CR, Cameron CE (2018) Functional insights from proteome-wide structural modeling of *Treponema pallidum* subspecies pallidum, the causative agent of syphilis. BMC Struct Biol 18(1): 7.

Hui DS, Azhar EI, Kim YJ, Memish ZA, Oh MD, Zumla A (2018) Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis 18(8): e217-27.

Ikai A (1980) Thermostability and aliphatic index of globular proteins. J Biochem 88(6): 1895-1898.

Indriani R (2001) Antibody response and protection of inactivated-local isolate vaccine for infectious bronchitis in laying chicken. J IImTer Vet 6(2): 134–140.

Jespersen MC, Peters B, Nielsen M, Marcatili P (2017) BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 45(W1): W24–W29.

Jyotisha, Singh S, Qureshi IA (2020) Multi-epitope vaccine against SARS-CoV-2 applying immunoinformatics and molecular dynamics simulation approaches. J Biomol Struct Dyn 6: 1–17.

Kamens J (2015) The Addgene repository: an international nonprofit plasmid and data resource. Nucleic Acids Res 43(D1): D1152–D1157.

Kathwate GH (2022) *In silico* design and characterization of multi-epitopes vaccine for SARS-CoV2 from its spike protein. Int J Pept Res Ther 28(1): 37.

Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ (2015) The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10(6): 845–858.

Khalil I, Omer I, Farh IZ, Mohamed HA, Elsharif HA, Mohamed AA, Awad-Elkareem MA, Salih MA (2018) Design of an epitope-based peptide vaccine against *Cryptococcus neoformans*. BioRxiv [Preprint]. doi: https://doi.org/10.1101/434779

Ko J, Park H, Heo L, Seok C (2012) GalaxyWEB server for protein structure prediction and refinement. Nucleic Acids Res 40(W1): W294–W297.

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581(7807): 215–220.

Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet 395: 565–574.

Martínez-Flores D, Zepeda-Cervantes J, Cruz-Reséndiz A, Aguirre-Sampieri S, Sampieri A, Vaca L (2021) SARS-CoV-2 vaccines based on the spike glycoprotein and implications of new viral variants. Front Immunol 12: 701501.

McCartney S, Vermi W, Gilfillan S, Cella M, Murphy TL, Schreiber RD, Murphy KM, Colonna M (2009) Distinct and complementary functions of MDA5 and TLR3 in poly (I: C)-mediated activation of mouse NK cells. J Exp Med 206(13): 2967–2976.

McKee AS, Munks MW, Marrack P (2007) How do adjuvants work? Important considerations for new generation adjuvants. Immunity 27(5): 687–690.

Naveed M, Tehreem S, Arshad S, Bukhari SA, Shabbir MA, Essa R, Ali N, Zaib S, Khan A, Al-Harrasi A, Khan I (2021) Design of a novel multiple epitope-based vaccine: An immunoinformatics approach to combat SARS-CoV-2 strains. J Infect Public Health 14(7): 938–946.

O'Hagan DT, De Gregorio E (2009) The path to a successful vaccine adjuvant– 'the long and winding road'. Drug Discov Today 14(11-12): 541–551.

Oany AR, Ahmad SA, Hossain MU, Jyoti TP (2015) Identification of highly conserved regions in L-segment of Crimean–Congo hemorrhagic fever virus and immunoinformatic prediction about potential novel vaccine. Adv Appl Bioinform Chem 8: 1–10.

Oany AR, Emran AA, Jyoti TP (2014) Design of an epitope-based peptide vaccine against spike protein of human coronavirus: an in-silico approach. Drug Des Devel Ther 8: 1139–1149.

Olvera A, Noguera-Julian M, Kilpelainen A, Romero-Martín L, Prado JG, Brander C (2020) SARS-CoV-2 consensus-sequence and matching overlapping peptides design for COVID19 immune studies and vaccine development. Vaccines 8(3): 444.

Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z (2020) Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its

immune cross-reactivity with SARS-CoV. Nat Commun 11(1): 2144.

Phan AT, Goldrath AW, Glass CK (2017) Metabolic and epigenetic coordination of T cell and macrophage immunity. Immunity 46(5): 714–729.

Poran A, Harjanto D, Malloy M, Arieta CM, Rothenberg DA, Lenkala D, van Buuren MM, Addona TA, Rooney MS, Srinivasan L, Gaynor RB (2020) Sequence-based prediction of SARS-CoV-2 vaccine targets using a mass spectrometry-based bioinformatics predictor identifies immunogenic T cell epitopes. Genome Med 12(1): 70.

Prajapat M, Sarma P, Shekhar N, Avti P, Sinha S, Kaur H, Kumar S, Bhattacharyya A, Kumar H, Bansal S, Medhi B (2020) Drug targets for corona virus: A systematic review. Indian J Pharmacol 52(1): 56–65.

Raoult D, Zumla A, Locatelli F, Ippolito G, Kroemer G (2020) Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. Cell Stress 4(4): 66–75.

Reche PA, Fernandez-Caldas E, Flower DR, Fridkis-Hareli M, Hoshino Y (2014) Peptide-based immunotherapeutics and vaccines. J Immunol Res 2014: 256784.

Sadat SM, Aghadadeghi MR, Yousefi M, Khodaei A, Larijani MS, Bahramali G (2021) Bioinformatics analysis of SARS-CoV-2 to approach an effective vaccine candidate against COVID-19. Mol Biotechnol 63(5): 389–409.

Safavi A, Kefayat A., Mahdevar E, Abiri A, Ghahremani F (2020) Exploring the out of sight antigens of SARS-CoV-2 to design a candidate multi-epitope vaccine by utilizing immunoinformatics approaches. Vaccine 38(48): 7612–7628.

Sarma P, Shekhar N, Prajapat M, Avti P, Kaur H, Kumar S, Singh S, Kumar H, Prakash A, Dhibar DP, Medhi B (2021) In-silico homology assisted identification of inhibitor of RNA binding against 2019-nCoV N-protein (N terminal domain). J Biomol Struct Dyn 39(8): 2724–2732.

Shokeen K, Pandey S, Shah M, Kumar S (2020) Insight towards the effect of the multibasic cleavage site of SARS-CoV-2 spike protein on cellular proteases. BioRxiv [Preprint]. doi: https://doi.org/10.1101/2020.04.25.061507.

Singh A, Thakur M, Sharma LK, Chandra K (2020) Designing a multi-epitope peptide-based vaccine against SARS-CoV-2. Sci Rep 10(1): 16219.

Srivastava S, Kamthania M, Kumar Pandey R, Kumar Saxena A, Saxena V, Kumar Singh S, Kumar Sharma R, Sharma N (2019) Design of novel multi-epitope vaccines against severe acute respiratory syndrome validated through multistage molecular interaction and dynamics. J Biomol Struct Dyn 37(16): 4345–4360.

Tahir Ul Qamar M, Rehman A, Tusleem K, Ashfaq UA, Qasim M, Zhu X, Fatima I, Shahid F, Chen LL (2020a) Designing of a next generation multi-epitope-based vaccine (MEV) against SARS-COV-2: Immunoinformatics and *in silico* approaches. PloS One 15(12): e0244176.

Tahir Ul Qamar M, Shokat Z, Muneer I, Ashfaq UA, Javed H, Anwar F, Bari A, Zahid B, Saari N (2020b) Multiepitope-based subunit vaccine design and evaluation against respiratory syncytial virus using reverse vaccinology approach. Vaccines 8(2): 288.

Tahir Ul Qamar MT, Bari A, Adeel MM, Maryam A, Ashfaq UA, Du X, Muneer I, Ahmad HI, Wang J (2018) Peptide vaccine against chikungunya virus: Immuno-informatics combined with molecular docking approach. J Transl Med 16(1): 298.

Tahir Ul Qamar MT, Saleem S, Ashfaq UA, Bari A, Anwar F, Alqahtani S (2019) Epitope-based peptide vaccine design and target site depiction against Middle East Respiratory Syndrome Coronavirus: An immune-informatics study. J Transl Med 17(1): 362.

Wan Y, Shang J, Graham R, Baric RS, Li F (2020) Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS coronavirus. Virol J 94(7): e00127-20.

Weiss SR, Navas-Martin S (2005) Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev 69(4): 635–664.

WHO (2021) World Health Organization. https://covid19.who.int/ [Consulted September 27, 2021].

Wu JT, Leung K, Leung GM (2020) Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: A modelling study. Lancet 395(10225): 689–697.

Yang Z, Bogdan P, Nazarian S (2021) An *in silico* deep learning approach to multi-epitope vaccine design: A SARS-CoV-2 case study. Sci Rep 11(1): 3238.

Zeng L, Li D, Tong W, Shi T, Ning B (2021) Biochemical features and mutations of key proteins in SARS-CoV-2 and their impacts on RNA therapeutics. Biochem Pharmacol 189: 114424.

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579 (7798): 270–273.

Zhu X, Liu Q, Du L, Lu L, Jiang S (2013) Receptor-binding domain as a target for developing SARS vaccines. J Thorac Dis 5(Suppl 2): S142–S148.

AUTHOR CONTRIBUTION:

Contribution	Shehzad A	Sumartono C	Nugraha J	Susilowati H	Wijaya AY	Ahmad HI	Kashif M	Tyasningsih W	Rantam FA
Concepts or ideas	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x	x
Literature search	x	x	x			x		x	
Experimental studies	x	x	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x	x	x	x
Data analysis	x	x	x	x	x	x	x	x	x
Statistical analysis	x	x	x	x	x	x	x	x	x
Manuscript preparation	x	x	x	x	x	x	x	x	x
Manuscript editing	x	x	x					x	
Manuscript review	x	x	x	x	x	x	x	x	x

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