Frontiers in Dentistry

Vaccine Design against *Fusobacterium nucleatum* FadA Protein in Periodontal Infections

Neha N. Rathod[1], M. Jeevitha[1]*, Pradeep Kumar Yadalam[1], Amir Raee[2,3], Artak Heboyan[4,5,6]

1. Department of Periodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India
2. Dental Implant Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Periodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Research Analytics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
5. Department of Prosthodontics, Faculty of Stomatology, Yerevan State Medical University after Mkhitar Heratsi, Yerevan, Armenia
6. Department of Prosthodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

Article Info

Article type:
Original Article

Article History:
Received: 25 Mar 2025
Accepted: 20 Jul 2025
Published: 1 Apr 2026

**** Corresponding author:***
Department of Periodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India

Email: jeevitham.sdc@saveetha.com

ABSTRACT

Objectives: A common inflammatory condition affecting oral health is periodontitis, which can lead to tooth loss if not treated early, as it destroys the supporting structures of the teeth. *Fusobacterium nucleatum* is an orange-pigmented bacterium commonly referred to as "the bridging species" between early and late colonizers in the oral microbiota, and it is associated with the progression of periodontal disease. This study aimed to develop an effective and precise multi-epitope vaccine against *F. nucleatum*, as there is currently no approved vaccine for periodontal disease.

Materials and Methods: This study employed a computational method to design a multiple epitope-based vaccine against *F. nucleatum* using an immunoinformatics reverse vaccinology approach. FadA, a novel adhesin unique to *F. nucleatum*, was a suitable peptide for vaccine development. B- and T-cell epitopes were identified and assessed to design a multi-epitope vaccine. The developed vaccine construct was analyzed for potency based on its allergenicity, antigenicity, solubility, and stability.

Results: The vaccine structure was non-allergenic and exhibited high antigenicity, stability, and solubility. This vaccine may be a candidate for eradicating *F. nucleatum,* based on predictions of immunogenicity responses.

Conclusion: The designed vaccine construct could generate an effective immune response against *F. nucleatum,* owing to its high antigenicity and non-allergenicity.

Keywords: *Fusobacterium Nucleatum*; Periodontitis; Vaccination; Immunology

- **Cite this article as:** Rathod NN, Jeevitha M, Yadalam PK, Raee A, Heboyan A. Vaccine Design against *Fusobacterium nucleatum* FadA Protein in Periodontal Infections. *Front Dent.* 2026:23:15. http://doi.org/10.18502/fid.v23i15.21618

INTRODUCTION

Traditional vaccines were created with little to no understanding of how our immune systems are modulated. The vaccines were developed based on the pathogen's activity. Currently, immunoinformatics has helped improve understanding of microorganisms and disease processes. Genetic data are used in reverse vaccinology as a starting point for vaccine development [1]. Scanning the protein sequences of a pathogen of interest enables the identification of vaccine candidate epitopes using computational immunology and in in silico vaccine design tools. This technique has been employed in recent years to develop vaccines against the dengue virus [2], the Ebola virus [3], the avian influenza virus [4], and the coronavirus [5].

Fusobacterium nucleatum, a gram-negative bacilli found in the subgingival plaque, belongs to the orange complex that has been considered the "bridging species" between the early and late [6]colonizers and is necessary for bacterial interaction and coaggregation, leading to the maturation of plaque, which is the primary etiological factor of periodontitis [7–12]. It is becoming more prevalent in areas where periodontitis is present and is isolated from extra-oral infections such as those of the blood, lung, liver, joint, brain, chest, abdominal, obstetric and gynecological systems, including intrauterine infections, pregnancy complications such as preterm birth, low birth weight, and several abscesses [13]. *F. nucleatum*, a gram-negative bacterium found in the oral cavity, has been linked to colon cancer development [6]. It causes inflammation, damages the intestinal epithelial barrier, promotes tumor growth, and alters the tumor microenvironment [14]. It also stimulates immune evasion and inhibits the human immune response, potentially accelerating tumor growth. Targeting these bacteria could potentially prevent and treat colon cancer, though their exact roles are unclear [13]. It has been discovered that this adhesion protein plays a part in colorectal cancer by binding to E-cadherin on the surface of host cells and accelerating their growth. It has also been linked to respiratory and urinary tract infections, osteomyelitis, stomach cancer, esophageal cancer, and breast cancer [13]. Researchers have been investigating new immunogenic targets for *F. nucleatum* in the field of reverse vaccinology. This Gram-negative bacterium is found in the human oral cavity and is important in the development of periodontal disorders. The creation of a potent vaccination against this bacterium is crucial, given the growing resistance to antimicrobial drugs [15,16].

In this study, epitope-based vaccines against the adhesion protein FadA of *F. nucleatum* were created using various computational techniques. These vaccines activate cytotoxic T lymphocytes (CTLs) and helper T cells (HTLs). These strategies are superior to traditional vaccine development techniques in reducing the risk of allergic reactions. Previously, our team has conducted extensive research in this field, which has yielded promising results [17–19]. Methods are also quick, simple, and economical. Hence, the primary goal of this study is to minimize and ultimately eliminate the diseases caused by *F. nucleatum* by designing a multi-epitope-based vaccine against it.

MATERIALS AND METHODS

This study used a computational approach to develop an immunoinformatics reverse vaccinology vaccine against *F. nucleatum* that uses multiple epitopes. FadA, a novel adhesin found only in *F. nucleatum*, was an appropriate peptide for vaccine development. B- and T-cell epitopes were identified and analyzed to develop a multi-epitope vaccine. The ethics code was not necessary for this study design.

Workflow:

The workflow for developing a multi-epitope vaccine against *F. nucleatum* involves retrieving the amino acid sequence, screening the N protein for antigenicity, predicting cytotoxic T-lymphocyte epitopes, predicting toxicity, using the consensus technique, and predicting helper T-lymphocyte epitopes. The vaccine combines adjuvant, CTL, MHC (Major Histocompatibility complex) Class I and II epitopes, and required linkers. The vaccine's physicochemical characteristics are assessed using the ExPASy server (http://web.expasy.org/protparam/), and molecular docking analysis is performed to evaluate the interaction between the vaccine candidate and the host immune receptor. The vaccine's allergenicity, antigenicity, solubility, and stability are assessed, and the structure prediction is validated.

Sequence retrieval of F. nucleatum using FadA adhesin protein

F. nucleatum's FASTA amino acid sequence was obtained using the PDB ID: 3ETW. The bacterial N protein was then screened for average antigenicity using Vaxijen (http://www.ddg-pharmfac.net/vaxijen/).

The immunogenicity of MHC-I epitopes was determined using the Immune Epitope Database (IEDB) server, which identifies peptide-MHC complexes recognized by T cells. The VaxiJen v2.0 server assessed the antigenicity of a multi-epitope vaccine sequence using a threshold of 0.4 for bacterial epitopes. The AllerTOP v2.0 server (https://www.ddg-pharmfac.net/AllerTOP/) evaluated allergic reactions caused by epitopes, while the ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/) checked epitope toxicity. These analyses were repeated after the vaccine was constructed.

Prediction of epitopes

NetCTL1.2 (http://www.cbs.dtu.dk/services/NetCTL/) was used to predict CTL epitopes for the N protein, using a threshold of 0.75, with 0.97 specificity and 0.80 sensitivity. The default parameters are C-terminal cleavage and Transporter Associated with Antigen Processing (TAP) transport efficiency.

The CTL epitopes for the N protein were predicted using NetCTL1.2 (http://www.cbs.dtu.dk/services/NetCTL/) with a threshold of 0.75. The toxicity of the antigens was predicted. With a percentile rank below 2, the consensus technique was used to predict MHC-I binding alleles for a subset of CTL epitopes using the IEDB server's MHC-I binding predictions (http://tools.iedb.org/mhci/). Helper T-lymphocyte (HTL) epitopes, which are 15 amino acids in length, were found using the IEDB MHC-II epitope prediction tool (http://tools.iedb.org/mhcii/), which uses the NN Align method to compute the percentile rank and IC50 value for the interaction of the peptide with MHC-II. Humans served as the source species; therefore, the study investigated the Human Leukocyte Antigen (HLA) loci, namely HLA-DR, HLA-DP, and HLA-DQ. The percentile ranks of less than 10nM and 1.5, respectively, and the IC50 values of less than 10nM were considered for prediction because these values signify stronger binding affinities. The antigenic qualities of predicted HTL epitopes were evaluated. The AllerTOP v2.0 server was used to assess whether specific epitopes elicit allergic reactions. The IEDB population coverage tool analyzes MHC restriction and population coverage of anticipated epitopes in different regions, calculating potential responses.

Creation of multi-epitope vaccine

The vaccine, which contained sufficient space for epitopes in vivo, was produced by combining the adjuvant, CTL, MHC Class I and II, and the required linkers. The EAAAK linker was used to connect epitopes to B-cell epitopes. Similarly, CTL, MHC Class I, and Class II epitopes were joined using GSGSGS, GSGSGS, and AAY linkers.

Evaluation of multi-epitope vaccine

Expasy server (http://web.expasy.org/protparam/) was used to model the identified epitope sequence to ascertain the physicochemical characteristics of the constructed vaccine.

Structure prediction and validation

Expasy (http://web.expasy.org/protparam/) was used to analyze the secondary structure content of the developed vaccine. The Expasy tool is used to analyze the secondary structure content of a vaccine by inputting the amino acid sequence into the Protparam tool. The tool uses predictive algorithms to estimate content using statistical and machine-learning techniques. This information helps understand the vaccine's structural characteristics, potential interactions with immune receptors, and overall conformation, aiding in the development and optimization of a successful multi-epitope vaccine against *F. nucleatum.*

Molecular docking analysis

To evaluate the interaction between the vaccine candidate and the host immune receptor, molecular docking was performed using the ClusPro 2.0 server (https://cluspro.bu.edu/publications.php), with the vaccine construct and insulin-derived growth factor (PDB ID 3ETW) as the receptor and ligand, respectively. Hence, rigid-body docking, clustering of the lowest-energy structures, and structural refinement were sequentially applied to generate complexes.

RESULTS

Antigenicity and allergenicity are key concepts in vaccine development and understanding pathogen recognition. Toxicity refers to harmful effects on living organisms, assessed by safe exposure levels. Table 1 contains predicted sequences related to toxicity, MHC class I and II, and CTL, suggesting potential immunological relevance. Antigenicity and allergenicity are crucial concepts in vaccine development and understanding pathogen recognition. Toxicity refers to harmful effects on living organisms, assessed by safe exposure levels. Predicted sequences related to toxicity, MHC class I and II, and CTL suggest potential immunological relevance. Fusobacterium adhesin shows good antigenicity, with a score of 0.5423, and is likely non-antigenic and non-allergenic. All predicted epitopes are nontoxic, with four peptides having negative SVM scores.

Peptides have been predicted as potential cytotoxic T-cell lymphocyte epitopes, with ELQALDAEY, ALAQNEQVY, QAEANTRFY, and TRFYKSQYQ demonstrating characteristics that make them likely to be recognized by cytotoxic T cells and elicit an immune response against specific targets. MHC Class I predicted epitopes exhibit high predicted binding affinity for their HLA-DRB alleles, suggesting a potential role in immune responses and antigen presentation.

The predicted protein structure of the vaccine construct from the Expasy server was validated using Ramachandran plots, revealing stability and correct folding. Protein-protein docking of FaDA with E-cadherin showed a strong interaction, suggesting their involvement in cell adhesion, signal transduction, or regulatory processes. The most effective vaccine was selected from the docked complexes with the lowest energy score and the energy between the receptor and ligand (Figs. 1,2,3).

Antigenicity, allergenicity, and toxicity were reported in this study and the predicted sequences of toxicity, MHC class I and II, and CTL were shown in Table 1.

The Vaxign tool predicted a probable antigen that could potentially confer protection against *F. nucleatum* infection. Based on the results obtained from the allertop tool, a probable non-allergenic antigen derived from *F. nucleatum* was also predicted. This means the identified antigen is unlikely to cause an allergic reaction in individuals.

Table 1 predicts the toxicity of the antigen, reporting that all predicted epitopes are nontoxic. Each peptide is described by its sequence, SVM score, prediction, hydrophobicity, hydropathicity, and hydrophilicity.

The SVM score indicates non-toxicity, while hydrophobicity, hydropathicity, and hydrophilicity values indicate their physicochemical properties. All four peptides have negative SVM scores, indicating non-toxicity. The SVM score, a machine learning algorithm, predicts peptide toxicity based on hydrophobicity, hydropathicity, and hydrophilicity values. All four peptides have negative scores, indicating non-toxicity in their potential physiological roles.

Table 2 shows sequences that have been predicted as potential cytotoxic T-cell lymphocyte epitopes: ELQALDAEY, ALAQNEQVY, QAEANTRFY, and TRFYKSQYQ. These sequences demonstrate characteristics that make them likely to be recognized by cytotoxic T cells and could elicit an immune response against specific targets.

Table 1. Predicted toxicity of the antigen

Peptide ID. (Seq)	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity
Seq1 (ELQALDAEY)	-0.88	Non-Toxin	-0.12	-0.46	0.26
Seq2 (ALAQNEQVY)	-1.16	Non-Toxin	-0.12	-0.41	-0.33
Seq3 (QAEANTRFY)	-0.86	Non-Toxin	-0.31	-1.18	0.02
Seq4 (TRFYKSQYQ)	-0.72	Non-Toxin	-0.44	-1.86	-0.09

Seq: Sequence; SVM: Support Vector Machine.

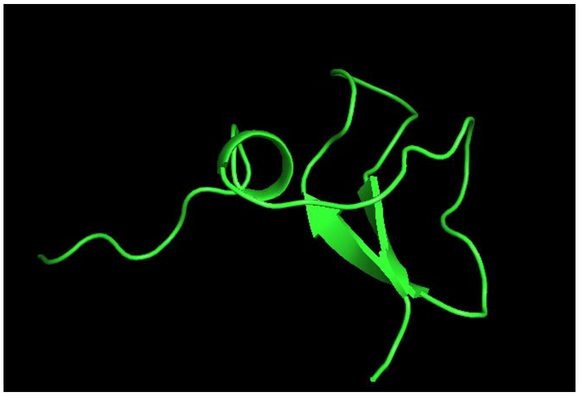

Fig 1. Predicted protein structure of the vaccine construct from the ExPASy server. The Expasy server was utilized to predict the protein structure of the vaccine construct. Predicted protein structure refers to the multi-epitope vaccine construct designed from the FadA adhesin protein of *Fusobacterium nucleatum*.

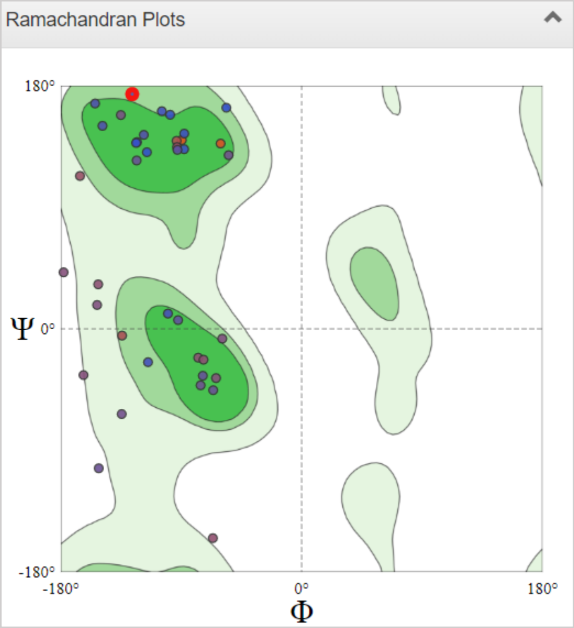

Fig 2. Validation of predicted vaccine construct using Ramachandran plot showing allowed regions. The Ramachandran plot analyzes protein structures' dihedral angles, revealing allowed and disallowed regions. In the vaccine construct, most dihedral angles fall within allowed regions, indicating stability and correct folding. This is crucial for vaccine quality and effectiveness, promoting an effective immune response.

Table 3 shows MHC Class I predicted epitopes (top 5 based on IC50), reporting the predicted likelihood of peptides being recognized by cytotoxic T cells as potential epitopes. Scores are calculated using computational algorithms that consider factors such as binding affinity for MHC-I molecules.

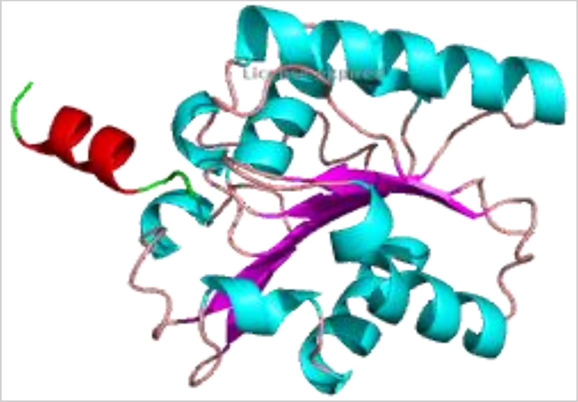

Fig 3. Molecular docking of FadA with E-cadherin using CLUSPRO 2.0. The binding energy of -306.9 kJ·mol−1 indicates a strong interaction between FadA and E-cadherin during protein-protein docking. This indicates a strong affinity between them and a stable complex formation. This strong interaction could have significant biological implications, suggesting their involvement in cell adhesion, signal transduction, or regulatory processes. Thus, FadA and E-cadherin may have important biological roles in the same pathway or functional module.

Table 2. Predicted cytotoxic T cell lymphocyte epitopes

Number	Epitope sequence
1	ELQALDAEY
2	ALAQNEQVY
3	QAEANTRFY
4	TRFYKSQYQ

Table 3. Major Histocompatibility Complex (MHC) Class I predicted epitopes (top 5 based on IC50)

Peptide	Length	Score
QAEANTRFY	9	0.21419
ELQALDAEY	9	0.10337
ALAQNEQVY	9	-0.08955
TRFYKSQYQ	9	-0.2793

IC50: Half Maximal Inhibitory Concentration.

A higher score indicates a higher probability of binding to MHC-I molecules and being presented on infected or cancerous cells. QAEANTRFY has the highest score of 0.21419, followed by ELQALDAEY at 0.10337, ALAQNEQVY at -

0.08955, and TRFYKSQYQ at -0.2793. The global population coverage analysis for the eight selected epitopes indicates that 55% of people are predicted to respond to the CTL epitopes. Similarly, the analysis reveals that 27% of people are predicted to respond to the HTL epitopes.

Table 4 reveals that peptide sequences exhibit high predicted binding affinity for their HLA-DRB alleles, suggesting a potential role in immune responses and antigen presentation. The percentile rank, combilib score, and combilib rank provide information about the predicted binding affinity. A lower percentile rank indicates a greater likelihood that the peptide sequence will be presented on the cell surface, potentially triggering an immune response. Further analysis and experimentation are needed to determine the specific characteristics and functional implications of these peptide sequences.

The Ramachandran plot analyzes protein structures' dihedral angles, revealing allowed and disallowed regions. In the vaccine construct, most dihedral angles fall within allowed regions, indicating stability and correct folding. This is crucial for vaccine quality and effectiveness, promoting an effective immune response.

The binding energy of -306.9kJ/mol indicates a strong interaction between FadA adhesion-derived vaccine and E-cadherin receptor during protein-protein docking (Table 5).

Table 4. Major Histocompatibility Complex (MHC) Class II predicted (top 5 based on IC50)

Allele	Start	End	Peptide	Percentile Rank	Comblib Score	Comblib Rank
HLA-DRB1*07:01	98	112	ISDFEKIQALRAGNU	4.3	289.32	39
HLA-DRB1*07:01	99	113	SOFEKIQALRAGNLE	4.4	289.32	39
HLA-DRB3*01:01	55	69	QRAQRIQAEANTRY	4.6	552.7	36
HLA-DRB3*01:01	57	71	AQRLQAEANTRPYKS	4.7	552.7	36
HLA-DRB3*01:01	56	70	RAQRLQAEANTRIVK	4.7	552.7	36

IC50: Half Maximal Inhibitory Concentration; Comblib Score: combinatorial library score; Comblib Rank: combinatorial library Rank.

Table 5. Cluster score of the vaccine with its center and lowest energy

Cluster	Members	Representative	Weighted Score
0	449	Center	-306.9
		Lowest Energy	-361.7
1	298	Center	-288.0
		Lowest Energy	-331.7
2	95	Center	-314.8
		Lowest Energy	-326.2
3	84	Center	-295.8
		Lowest Energy	-321.3
4	43	Center	-287.6
		Lowest Energy	-318.9
5	20	Center	-306.2
		Lowest Energy	-306.2
6	7	Center	-288.5
		Lowest Energy	-300.3
7	3	Center	-289.5
		Lowest Energy	-292.4

This indicates a strong affinity between them and a stable complex formation. This strong interaction could have significant biological implications, suggesting their involvement in cell adhesion, signal transduction, or regulatory processes. Thus, FadA and E-cadherin may have important biological roles in the same pathway or functional module.

Binding energy shows a very strong interaction of FadA with E-cadherin in the lowest binding energy of -306.9kJ/mol. It shows the predicted vaccine structure and its validation using Ramachandra plots. The interaction of vaccine epitopes (FadA) with E-cadherin was examined using molecular docking on the ClusPro 2.0 server (Figs. 1, 2, and 3). The most effective vaccine was selected from the docked complexes with the lowest energy score (-361.7kJ/mol) and the energy between the receptor and ligand (center energy -306.9kJ/mol).

DISCUSSION

The discipline of immunoinformatics vaccine design employs bioinformatics tools and computational techniques to select the most promising vaccine candidates. Antigen selection, epitope prediction, immunogenicity prediction, and in in silico evaluation are critical steps in this procedure. Immunoinformatics thereby streamlines vaccine production through computational approaches. It is essential to identify potential antigens, select the most immunogenic epitopes, and develop vaccines that elicit a strong, focused immune response. In this study, an immunogenic multi-epitope vaccine against *F. nucleatum* was developed using immunoinformatics to control and ultimately eradicate periodontitis. The epitopes from *F. nucleatum* were chosen based on their antigenicity, immunogenicity, non-allergenicity, and number of MHC class I and II binding alleles. The vaccine candidate contains epitopes for helper T lymphocytes (HTL), cytotoxic T lymphocytes (CTL), and B cells (BCL), all of which are essential for inducing antibody production. To confirm the vaccine candidate's binding mechanism with the host immune receptor, molecular docking was used to design a unique, multi-epitope vaccine candidate that could lead to a reliable periodontitis vaccine. Stimulation of cytotoxic T-cells induces plasma cells to produce antibodies. Moreover, helper T-lymphocytes target infected cells and destroy them, aided by these antibodies through B-cell stimulation. Thus, analysis of the helper T-lymphocyte (HTL) epitope is crucial. HTL epitopes were predicted using IC50 values (10nM) and percentile rank (1.5) for the HLA-DR, HLA-DQ, and HLA-DP loci.

Several criteria were satisfied for the HTL epitopes on the HLA-DR gene. The HTL epitope that constitutes CTL, MHC-I, and MHC-II (tables 1, 2, 3, and 4) was selected and used in the vaccine's development. The developed multi-epitope vaccine construct underwent molecular docking, which showed that it was non-allergenic, soluble, stable, and had the lowest binding energy (361.7kJ/mol) (figs. 2, 3). This shows that the vaccine design can be subjected to subsequent development and testing for vaccination against the periodontal pathogen *F. nucleatum.*

Studies in the past have used the reverse vaccinology approach to develop vaccines against several diseases. In their study, PK Yadalam et al. used the major fimbrial protein and an immunoinformatic reverse vaccinology approach to develop a vaccine for peri-implantitis [17]. Immunoinformatics was used to design a vaccine targeting the SARS-CoV-2 nucleocapsid protein [18]. A multi-epitope vaccine against EBOV was developed by Alizadeh et al. using immunoinformatics tools [19].

When intermediate colonizers such as *F. nucleatum* and late colonizers such as Treponema denticola recognize polysaccharide or protein-binding sites on the cell surface of primary colonizers, the dental plaque biofilm begins to mature. As a result, primary colonizing bacteria such as Neisseria and Streptococcus are reduced in number, while the proportions of immediate and late colonizers in the dental plaque biofilm rise. Ninety percent of dental biofilm-associated bacteria have been found to have a paired-aggregation tendency. The receptor sites of each bacterial species are selective for complementary binding to the adhesion molecules of certain bacteria; therefore, this coaggregation is not random. As an example, *F. nucleatum* and Streptococcus mutans coaggregate, although the latter cannot attach to Porphyromonas gingivalis, which is considered a key pathogen in periodontal diseases. Another characteristic of some bacterial species is coaggregation, in which distinct receptor sites can bind to two or more other bacterial species. The most well-known bridging species is *F. nucleatum*, an orange-pigmented bacterium that facilitates coaggregation between strictly anaerobic and aerobic bacteria. Mature dental biofilm bacterial coaggregation has distinct patterns, such as "corn on the cob" structures and "bristle brush formations." [16]. As a result of this study, *F. nucleatum* may now be the target of strong memory reactions and humoral immune responses. This can significantly slow the spread of periodontitis, enhancing dental and general public health.

Future research could include in vivo animal studies to assess the immune response and potential side effects. Clinical trials involving human subjects could assess the vaccine's effectiveness in reducing the severity of periodontitis. Optimizing vaccine formulation and delivery methods could enhance immunogenicity and shelf life. Limitations include in silico analysis and molecular docking, which should be validated experimentally. Global population coverage analysis should consider HLA polymorphisms and potential challenges in development and implementation, such as regulatory approvals, manufacturing scale-up, cost-effectiveness, and public acceptance.

CONCLUSION

Using an immunoinformatics-based reverse vaccinology approach, a multi-epitope vaccine containing both B-cell and T-cell epitopes was designed from the adhesin protein FadA of *F. nucleatum*. The vaccine construct exhibited high antigenicity, solubility, and stability while remaining non-allergenic. Consequently, it is capable of eliciting both humoral and cell-mediated immune responses against *F. nucleatum*, which may help reduce its prevalence and aid in disease prevention.

ACKNOWLEDGEMENT

We want to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, and Saveetha University for their support of our study. The present project is supported by:
-Saveetha Institute of Medical and Technical Sciences
-Saveetha Dental College and Hospitals, Saveetha University

CONFLICT OF INTEREST STATEMENT

None declared.

GENERATIVE AI IN SCIENTIFIC WRITING

The authors declare that no generative artificial intelligence tool was used for data generation, analysis, interpretation, or scientific decision-making. Language editing support, if any, was limited to improving grammar and readability, and all content was critically reviewed and approved by the authors.

REFERENCES

1. Martinelli DD. In silico vaccine design: A tutorial in immunoinformatics. Healthcare Analytics. 2022 Nov 1;2:100044.
2. Ali M, Pandey RK, Khatoon N, Narula A, Mishra A, Prajapati VK. Exploring dengue genome to construct a multi-epitope based subunit vaccine by utilizing immunoinformatics approach to battle against dengue infection. Sci Rep. 2017 Aug 23;7(1):9232.
3. Bazhan SI, Antonets DV, Karpenko LI, Oreshkova SF, Kaplina ON, Starostina EV, et al. In silico Designed Ebola Virus T-Cell Multi-Epitope DNA Vaccine Constructions Are Immunogenic in Mice. Vaccines (Basel). 2019 Mar 29;7(2):34.
4. Hasan M, Ghosh PP, Azim KF, Mukta S, Abir RA, Nahar J, et al. Reverse vaccinology approach to design a novel multi-epitope subunit vaccine against avian influenza A (H7N9) virus. Microb Pathog. 2019 May;130:19-37.
5. Enayatkhani M, Hasaniazad M, Faezi S, Gouklani H, Davoodian P, Ahmadi N, et al. Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19: an in silico study. J Biomol Struct Dyn. 2021 May;39(8):2857-2872.
6. McIlvanna E, Linden GJ, Craig SG, Lundy FT,

James JA. Fusobacterium nucleatum and oral cancer: a critical review. BMC Cancer. 2021 Nov 13;21(1):1212.

7. Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ Jr. Communication among oral bacteria. Microbiol Mol Biol Rev. 2002 Sep;66(3):486-505, table of contents.

8. Sahu D, Kannan GM, Vijayaraghavan R. Carbon black particle exhibits size dependent toxicity in human monocytes. Int J Inflam. 2014;2014:827019.

9. Shebi S, Ezhilarasan D, Thomas J, Chandrasekaran N, Mukherjee A. Gracilaria foliifera (Forssk.) Børgesen Ethanolic Extract Triggers Apoptosis via Activation of p53 Expression in HepG2 Cells. Pharmacognosy Magazine. 2019 Mar 1;15(61).

10. Preethi KA, Sekar D. Dietary microRNAs: Current status and perspective in food science. J Food Biochem. 2021 Jul;45(7):e13827.

11. Bakshi HA, Mishra V, Satija S, Mehta M, Hakkim FL, Kesharwani P, et al. Dynamics of Prolyl Hydroxylases Levels During Disease Progression in Experimental Colitis. Inflammation. 2019 Dec;42(6):2032-2036.

12. Thakur RS, Devaraj E. Lagerstroemia speciosa (L.) Pers. triggers oxidative stress mediated apoptosis via intrinsic mitochondrial pathway in HepG2 cells. Environ Toxicol. 2020 Nov;35(11):1225-1233.

13. Chen Y, Huang Z, Tang Z, Huang Y, Huang M, Liu H, et al. More Than Just a Periodontal Pathogen - the Research Progress on Fusobacterium nucleatum. Front Cell Infect Microbiol. 2022 Feb 3;12:815318.

14. Wu J, Li Q, Fu X. Fusobacterium nucleatum Contributes to the Carcinogenesis of Colorectal Cancer by Inducing Inflammation and Suppressing Host Immunity. Transl Oncol. 2019 Jun;12(6):846-851.

15. Padma S, Patra R, Sen Gupta PS, Panda SK, Rana MK, Mukherjee S. Cell Surface Fibroblast Activation Protein-2 (Fap2) of Fusobacterium nucleatum as a Vaccine Candidate for Therapeutic Intervention of Human Colorectal Cancer: An Immunoinformatics Approach. Vaccines (Basel). 2023 Feb 23;11(3):525.

16. Abdulkareem AA, Al-Taweel FB, Al-Sharqi AJB, Gul SS, Sha A, Chapple ILC. Current concepts in the pathogenesis of periodontitis: from symbiosis to dysbiosis. J Oral Microbiol. 2023 Apr 2;15(1):2197779.

17. Yadalam PK, Rengaraj S, Mugri MH, Sayed M, Porwal A, Alahmari NM, et al. Designing an immunoinformatic vaccine for peri-implantitis using a structural biology approach. Saudi J Biol Sci. 2022 Jan;29(1):622-629.

18. Khan MS, Khan IM, Ahmad SU, Rahman I, Khan MZ, Khan MSZ, et al. Immunoinformatics design of B and T-cell epitope-based SARS-CoV-2 peptide vaccination. Front Immunol. 2023 Jan 4;13:1001430.

19. Alizadeh M, Amini-Khoei H, Tahmasebian S, Ghatrehsamani M, Ghatreh Samani K, Edalatpanah Y, et al. Designing a novel multi-epitope vaccine against Ebola virus using reverse vaccinology approach. Sci Rep. 2022 May 11;12(1):7757.