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Original article

Designing an immunoinformatic vaccine for *peri*-implantitis using a structural biology approach

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ABSTRACT

Objectives: Peri-implantitis is a destructive inflammatory process that affects the soft and hard tissues around dental implants. *porphyromonas gingivalis*, an anaerobic gram-negative bacterium, appears to be the main culprit. Since there is no efficient and specific vaccine to treat *peri*-implantitis, the goal of our research has been to develop a multi-epitope vaccination utilizing an immunoinformatics approach that targeted P. gingivalis type I fim A.

Materials and methods: *P. gingivalis* peptides 6JKZ and 6KMF are suitable for vaccine development. B- and T-cell epitopes from 6KMF and 6JKZ were detected and evaluated based on critical factors to produce a multi-epitope vaccine construct. It was assessed based on allergenicity, antigenicity, stability. The vaccine's dual major histocompatibility complex (MHC-I and MHC-II) binding epitopes allowed it to reach a larger population. *P. gingivalis* fimbriae induce immune subversion through TLR -CXCR4 receptor complex pathway. The ClusPro 2.0 server was used to do the molecular docking using TLR2 - CXCR4 and vaccine epitopes as receptor and ligand respectively.

Results: The designed vaccine was non-allergenic and had a high antigenicity, solubility, and stability. The 3D structure of the vaccine revealed strong interaction with CXCR4(TLR2) using molecular docking. The vaccine-CXCR4 interface was more consistent, possibly because the vaccination has a higher affinity for the CXCR4-TLR2 complex.

Conclusion: This study details the vaccine's distinct and sustained interaction with the CXCR4(TLR2) immunological receptor and its consistent and effective utterance in the bacterial system. As a result, our vaccine formulation will evoke a significant memory response and induce an adaptive immune response against *P. gingivalis*.

1. Introduction

Osseointegrated implants have been favored by clinicians in treating dentition defects and edentulism. Implants demonstrate predictable long-term stability and survival rates. However, there are reports of implant failures and post-implantation complications. The biological failures of implants can be divided based on chronology into early and late implant failures. Early failures refers to the lack of osseointegration. It can be a result of a difficult surgical technique, implant and patient-related factors. Late failures refer to the failure to maintain the achieved osseointegration

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1319-562X/

(Sakka et al., 2012). The most common reasons for late failures are prosthetic overloading or *peri*-implant illness (Mombelli and Lang, 1998).

Clinical and experimental research shows evidence that *peri*-implant illness is a key factor implicated in implant failure. Recent reports state the prevalence of *peri*-implant illness as 50% of implants placed (Zitzmann and Berglundh, 2008). There is an etiologic association between *peri*-implant illness and bacterial infection. Pathogenic microorganisms are the most common reason for *peri*-implant illness, categorized into *peri*-implant mucositis and *peri*-implantitis (Mombelli and Lang, 1998). Peri-implant mucositis is closely related to gingivitis in terms of it being a reversible inflammatrory condition. Peri-implantitis, however, closely corresponds to adult periodontitis with inflammation and loss of supporting bone around the dental implant. Paster et al found that a host of different organisms - *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans,* and *Prevotella intermedia* - all make a substantial contribution to the formation of deep periodontal pockets (Paster et al., 2001). These pathogens can impregnate the periodontal pockets associated with periodontitis and in the *peri*-implant region. Bacterial pathogens invade the *peri*-implant crevices within two weeks of implant placement (“Microbiota around root-form endosseous implants: A review of the literature,” 2003). Peri-implant illness and progression of *peri*-implant inflammation are further aggravated by plaque deposition at the implant site (Mombelli and Lang, 1998). Sustainable *peri*-implant sulci have pathogens that are similar to microorganisms present in good periodontal tissue. The bacteria detected in *peri*-implant disease are comparable to those seen in subgingival bacterial complexes of periodontitis patients. *P. gingivalis* and *A. actinomycetmcomitans* were discovered in the gingival crevices of implant abutments (de Oliveira et al., 2012). The microbial composition of early plaque around implants is also influenced by the state of the remaining teeth (Mombelli et al., 1995).

Porphyromonas gingivalis is a gram-negative anaerobic periodontal pathogen causes periodontal disease in the natural dentition. Research has implicated *P. gingivalis* with the deterioration of *peri*-implant tissue (Pérez-Chaparro et al., 2009). Salcetti et al found that failed implants had a higher prevalence of *P. gingivalis*, *T. forsythia*, and *T. denticola* species than healthy implants (Salcetti et al., n.d.). Botero et al examined the microorganisms present in healthy *peri*-implant tissue compared to those found in *peri*-implantitis affected tissue. They observed that *P. gingivalis* was only present in the diseased tissue (Botero et al., 2005).

P. gingivalis virulence factors incorporate fimbriae**,** capsule, collagenase, and gingipains (Amano, 2003; Ishikawa et al., 1995). The fimbriae of *Porphyromonas gingivalis* are critical for bacterial adhesion to the host cell, permitting pathogenic encroachment and contagion (Amano, 2003; Amano et al., 1994; Nakagawa et al., 2002a). Additionally, they promote early plaque accumulation and regulate plaque maturation (Enersen et al., 2013). The fimbria expresses numerous pro-inflammatory cytokines (IL-1, IL-6, and TNF-beta) that promote alveolar bone loss (Hamada et al., 2002). Lee et al were the first to describe genetic variations in the Fimbriae A protein (Lee et al., 1991). Nakagawa et al detected six distinct fimA genotypes (types I–V, Ib), which express fimbrillin, a fimbriae subunit (Nakagawa et al., 2002b). According to Amano et al, periodontal disease was substantially attributed to *P. gingivalis* with type I fimA (Zhao et al., 2007). Nagano et al discovered a positive correlation between fimA expression and plaque deposition across several genotypes, with type I fimA exhibiting a solid correlation with plaque formation (Nagano et al., 2013). Shin and Seo et al investigated the prevalence of *P. gingivalis* fimA genotypes in *peri*-implant crevices. They discovered that *P. gingivalis* type II fimA was strongly associated with *peri*-implantitis (Kim et al., 2016). However, this result must be viewed bearing in mind that cross-hybridization is a possibility during PCR analysis because type Ib and type II fimA share 97.1 and 77.5 percent of their nucleotide sequences (Nakagawa et al., 2002c; Enersen et al., 2008).

Sung-Geun Kim et al analyzed the association of fimA genotype in *peri*-implantitis based on probing depth. They found that Type Ib was present in 8.9% of specimens with a pocket depth of less than 5 mm. However, they were found in a greater percentage (21.4%) of specimens that had a pocket depth greater than 5 mm. Thus, type Ib fimA was associated with the progressive deepening of the probing depth during the progression of implant disease. The fimA type Ib genotype of *Porphyromonas gingivalis* was detected to be crucial for *peri*-implant tissue destruction, implying that it might be a potential cause for *peri*-implantitis (Kim et al., 2016)**.** Prevention of periimplantitis include Regular tooth brushing, interdental aids, chemical mouth rinse and regular followup.

Vaccination develops the immune system’s specific resistance to a particular bacterial or viral infection. When an individual develops immunity or resistance to infection following a secondary response (booster), the individual is considered immune to the disease. The first step in vaccine development is identifying an antigenic component from various organisms that can confer immunity. Antigens of pathogenic bacteria and viruses have been used to develop vaccines against many infectious diseases (Kaur, 2014).

Recent improvements in digital technology and computing provide an alternative for traditional antigenic epitope design with translational results. An immunoinformatics method was employed to generate an immunologic multi-epitope vaccine for *peri*-implantitis. The vaccine candidate contains T - helper (HTL), cytotoxic T-lymphocyte (CTL), and B-cell lymphocyte (BCL) epitopes which are critical for antibody generation. Molecular docking was used to validate the vaccine candidate’s association, binding mechanism, and reliability with the human host’s immune receptor CXCR4 to develop an innovative and potentially multi-epitope vaccine that could pave the way for a *peri*-implantitis vaccine (Kumar et al., 2021). We prioritised a group of epitopes using a computer-aided technique based on sequence conservation criteria and biological properties of their antigens of origin.

CXC-chemokine receptor 4 (CXCR4) and growth differentiation factor 5 (GDF5) cluster with TLR2-associated receptors interacting with Pg-fimbriae. The implementation of the two receptors was investigated. Long fimbriae were demonstrated to activate nuclear factor-kB (NF-kB) through the TLR 2 and CD14, resulting in the production of bone-resorption-related cytokines such as tumor necrosis factor-a (TNF-α), interleukin-1β (IL-1), IL-8, and IL-6 (Hajishengallis et al., 2006). Recent studies show that *P. gingivalis* can inhibit cell-mediated immunity by limiting interferon (IFN) growth and other cell-mediated immunity activators (Hajishengallis, 2011). Long fimbriae present in human monocytes engage with CXCR4, a TLR2-related receptor, limiting TLR2 stimulation. Long fimbriae also activate CAMP-dependent protein kinase A via CXCR4, reducing TLR2-induced NF-kB stimulation in exposure to *P. gingivalis*. These findings reveal that the lengthy fimbriae of *P. gingivalis* allow it to withstand clearance with a robust immune response both *in vivo* and *in vitro*, improving its optimized fitness. (Hajishengallis et al., 2008).

Vaccines have significantly reduced disease morbidity in most infectious diseases. Yet, vaccines in dentistry have met with only limited success.

This study explored the possibility of using major fimbrial protein to develop a vaccine against *peri*-implant diseases. We employed an immunoinformatics epitope vaccine approach to combat *peri*-implant disease-causing bacteria, particularly *P. gingivalis* serotype b. which shows promise as a vaccine candidate. This

```
RESULTS OF VACCINE
########################################
# Program: water
# Rundate: Tue 12 Jan 2021 05:23:26
# Commandline: water
#     -auto
#     -stdout
#     -asequence emboss_water-I20210112-052324-0697-42491664-p2m.asequence
#     -bsequence emboss_water-I20210112-052324-0697-42491664-p2m.bsequence
#     -datafile EBLOSUM62
#     -gapopen 10.0
#     -gapextend 0.5
#     -aformat3 pair
#     -sprotein1
#     -sprotein2
# Align_format: pair
# Report_file: stdout
########################################

#---------------------------------------
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 337
# Identity:       337/337 (100.0%)
# Similarity:     337/337 (100.0%)
# Gaps:             0/337 ( 0.0%)
# Score: 1756.0
#
```

Fig. 1. 6JKZ & 6KMF similarity using WATER TOOL software.

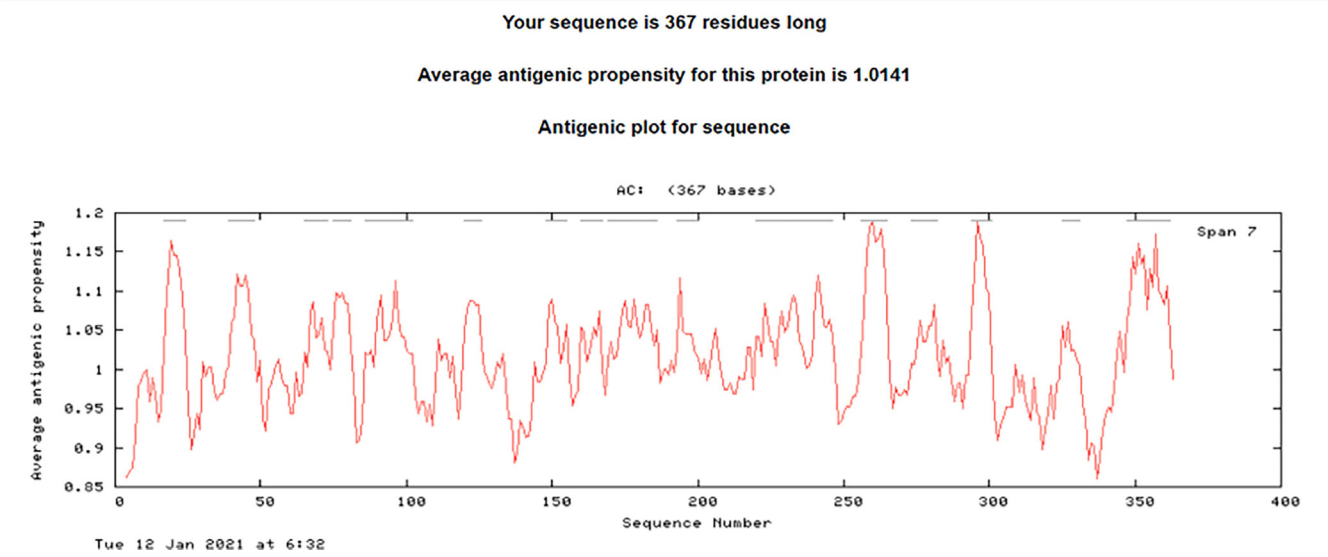

Fig. 2. The antigenic propensity of 6JKZ & 6KMF.

can help prevent *peri*-implantitis, bone loss and enhance implant survivablilty.

2. Materials and methods

2.1. Sequence analysis

The epitope peptide sequence of *P. gingivalis* was identified in Immune Epitope Database Analysis Resource with positive assays for linear epitopes. Identified peptide sequences were validated using the STRING tool. The network was built and analyzed for hubs, shortest path, and clustering coefficient.

The Amino acid sequence (FASTA) with the ID of **6JZK** and **6KMF**, belonging to *P. gingivalis*, was obtained from the PDB database**.** WATER TOOL software was used to achieve pairwise sequence alignment. 6JKZ and 6KMF were screened for average antigenic propensity and allergenicity using the **antigenic peptides prediction tool** (http://imed.med.ucm.es/Tools/antigenic.pl) and the **AllerTop v2.0 servers** (http://ddg-pharmfac.net/AllergenFP/) (Kumar et al., 2021).

2.2. Epitope prediction

The cytotoxic T-lymphocyte (CTL) epitopes for 6JZK and 6KMF were predicted using NetCTL1.2 (http://www.cbs.dtu.dk/services/NetCTL/) for all accessible serotypes with a threshold value of 0.75, a specificity of 0.97, and sensitivity of 0.80. Default settings of weight on C-terminal cleavage and TAP transport efficiency were maintained. Class I Immunogenicity of the IEDB server (http://tools.iedb.org/immunogenicity/) and VaxiJen v2.0 (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) were successively used to determine immunogenicity and antigenicity

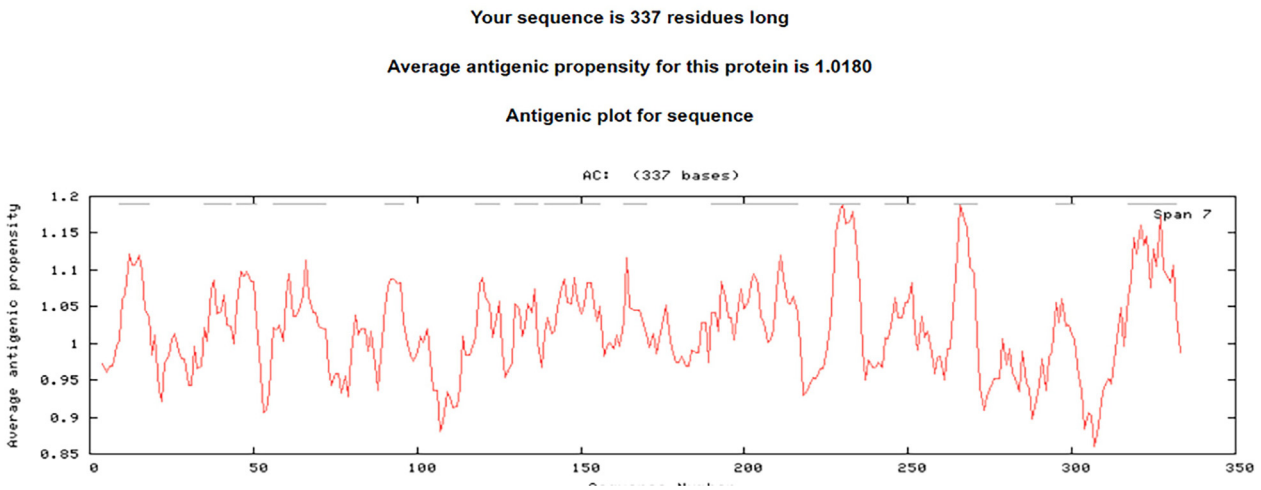

Fig. 3. The antigenic propensity of 6KMF.

Table 1
NetCTL-1.2 prediction using MHC supertype A1. Threshold 0.750000.

1	ID ID Sequence pep KTSNSNRAF aff 0.1388 aff_rescale 0.5893 cle 0.7978 tap 2.6640	C COMB 0.8422 < -E
2	ID Sequence pep VAKLTVMVY aff 0.1095 aff_rescale 0.4650cle 0.9766 tap 3.1380	COMB 0.7683 < -E
3	ID Sequence pep KAGKNYIGY aff 0.1200 aff_rescale 0.5096 cle 0.9636 tap 2.9690	COMB 0.8026 < -E
4	ID Sequence pep MSAAYDNIY aff 0.7480 aff_rescale 3.1759 cle 0.7830 tap 2.9800	COMB 3.4423 < -E
5	ID Sequence pep YTFVPEKIY aff 0.1703 aff_rescale 0.7232 cle 0.7824 tap 3.0040	COMB 0.9907 < -E
6	ID Sequence pep TLVNADANY aff 0.1317 aff_rescale 0.5592 cle 0.9420 tap 3.1550	COMB 0.8583 < -E
7	ID Sequence pep SLTTFNGAY aff 0.4751 aff_rescale 2.0172 cle 0.9263 tap 2.9310	COMB 2.3027 < -E
8	ID Sequence pep AADAPQGFY aff 0.5706 aff_rescale 2.4226 cle 0.8070 tap 2.8000	COMB 2.6837 < -E
9	ID Sequence pep YSANGGTIH aff 0.1870 aff_rescale 0.7939 cle 0.0376 tap −0.6370	COMB 0.7677 < -E
10	ID Sequence pep WVDAEGKTY aff 0.5142 aff_rescale 2.1832 cle 0.5043 tap 3.0010	COMB 2.4089 < -E
11	ID Sequence pep LAEVKALTTEaff 0.2888 aff_rescale 1.2260cle 0.9734 tap 3.0200	COMB 1.5230 < -E
12	ID Sequence pep ITESAHLNV aff 0.2741 aff_rescale 1.1640 cle 0.7765 tap 0.0270	COMB 1.2818 < -E

respectively (Kumar et al., 2021). The MHC-I binding alleles of selected CTL epitopes were identified using the MHC-I binding predictions of the IEDB server (http://tools.iedb.org/mhci/) using a conventional technique with a percentile rank of <2.

The IEDB MHC-II epitope prediction tool (http://tools.iedb.org/mhcii/)NN Align technique was used to obtain percentile rank and IC50 value peptide-MHC-II interactions. The source species was human. The loci HLA-DR, HLA-DP, and HLA-DQ were studied further. For prediction, IC50 values <10 nM and percentile rank <1.5 were used, as these values reflect stronger affinity. Antigenic characteristics of predicted HTL epitopes were evaluated.

Finally, the 6JKZ &6KMF epitopes from CTL, HTL, BCL were selected based on their allergenicity, toxicity, and antigenicity. The predicted epitope and one common epitope 6JKZ & 6KMF were used for multi-epitope vaccine constructions.

2.3. Molecular docking

For 6JKZ & 6KMF epitopes with CXCR4 (protein-peptide complex), molecular docking was performed using the ClusPro 2.0 (https://cluspro.bu.edu/publications.php) server. As a result, the complexes were created in three steps: rigid-body docking, lowest energy structure clustering, and structural refining.

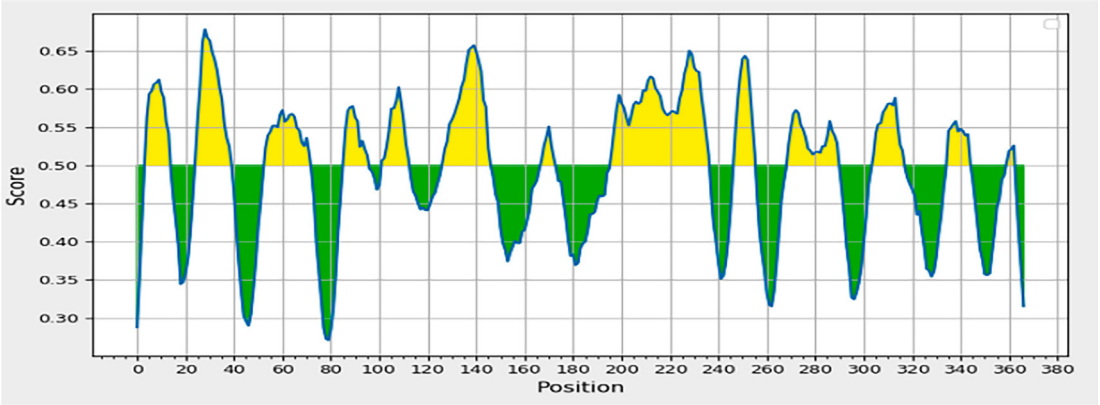

Fig. 4. BCL Epitope Confirmation with BepiPred for 6JKZ.

3. Results

3.1. Evaluation of P. gingivalis peptide sequences

The peptide sequence of *P. gingivalis* fimA type Ib **6JKZ** was aligned with its *P. gingivalis* fimA type Ib **6KMF** homolog using software WATER TOOL. The identity and similarity between these proteins were found to be 100% with zero penalties **(**Fig. 1**)**. The peptide of 6JKZ comprises 367 amino acids, and 6KMF consists of 337 amino acids with approximate molecular weight. Generally, proteins with an antigenic predicted score greater than 0.8 are evaluated for vaccine development utilizing epitope identification. The average antigenic susceptibility of 6JKZ and 6KMF was determined to be 1.0180 and 1.0141 respectively (Fig. 2 and Fig. 3). Further examination revealed that they were non-allergenic. 6JKZ and 6KMF were chosen for the development of epitope vaccination.

3.2. T-lymphocyte epitope prediction and assessment

Cytotoxic T-lymphocyte (CTL) epitopes are crucial for the stimulation of major histocompatibility complex immunological responses. Epitopes of JKZ and KMF were identified using the NetCTL1.2 service. From all MHC-I serotypes, 12 epitopes from 6JKZ and 6KMF with cumulative scores of >0.75 were found (Tables 1 and 2).

Cytotoxic T-cells are activated by Helper T-lymphocytes to generate antibodies and destroy infected target cells. Helper T-lymphocyte (HTL) epitopes for 6JKZ and 6KMF were estimated using the MHC-II epitope module of IEDB. HTL epitopes for 6JKZ &6KMF were predicted for HLA-DR, HLA-DQ, and HLA-DP loci based on IC50 values (<10 nM) and percentile rank (<1.5). The HTL epitopes of the HLA-DR locus satisfied various criteria. The HTL epitope (LAEVKALTTELTAEN) for 6JKZ and epitope for 6KMF (LAEVKALTTELTAEN) obtained from MHC-II were found to be similar. The HTL epitope (LAEVKALTTELTAEN) was selected as present in the C-terminal dimerization domain used for vaccine constructions (Table 3).

3.3. B-lymphocytes prediction and assessment

B-cells epitopes are an essential component for antibody formation. ABCPred (Table 4) was used to identify B-cell epitopes with a 0.5 or higher 16-mer length score, which was confirmed using the BepiPredserver (Figs. 4 and 5). BCL epitopes for both 6JKZ &6KMF (LAEVKALTTELTAEN) were found to fulfill both servers' parameters and were antigenic, non-allergenic, and non-toxic.

Finally, the one common epitope of 6JKZ & 6KMF (LAEVKALTTELTAEN) was selected based on prediction and analysis of CTL, HTL &BCL for vaccine construction.

3.4. Molecular docking

ClusPro 2.0 server was for molecular docking to examine the interaction of vaccination epitopes with TLR2 receptorCXCR4 (Figs. 6 and 7). The best vaccination CXCR4 complex was chosen from the docked complexes with the lowest energy score (595.4 kJ mol1) and the energy between receptor and ligand (center energy − 493.2 kJ mol^{-1}**)**. **(**Fig. 8**)**.

4. Discussion

The primary objective of any periodontal vaccine is to reduce periodontal disease with the ultimate aim of eradicating periodontal illness. *Porphyromonas gingivalis* is an etiological agent linked to periodontal illnesses. Most notably, the type Ib genotype has been linked to affected *peri*-implant tissue and may play an active role in its destruction.

Immunoinformatics has been frequently used to produce innovative and effective *Porphyromonas gingivalis* epitope-based vaccines. *P. gingivalis* peptides 6JKZ & 6KMF are antigenic and non-allergenic. This indicates that they can elicit an immune response without generating any adverse effects, making them ideal candidates for vaccine development.

Epitopes stimulate cytotoxic T-lymphocytes and B-cell lymphocytes to eliminate pathogens via cytokine action. The cytokines signaled via the helper T-lymphocytes activate the immune system. CTL, HTL, and BCL epitopes were utilized to boost humoral and cell-mediated immunity because of their essential roles during the antibody response. The antigenicity, immunogenicity, non-allergenic and non-toxic nature, and the quantity of MHC-I and II binding alleles are all factors we considered when selecting epitopes from *P. gingivalis* 6JKZ & 6KMF peptides.

Table 4
Predicted B-cell epitope. A higher score of the peptide means a higher probability of being an epitope.

Peptide sequences	Start position	Score
TLVNADANYLTGSLTT	196	0.60
LAEVKALTTELTAENQ	94	0.56
VTEGNATISVVLKTSN	12	0.55
KLQKNGADLAGADLAA	266	0.51

Table 2
C. TL: MHC-I Prediction method: NetMHCpan EL 4.1 | High Score = good binder.

Alleles	#	start	End	length	Peptide	Core	icore	Score	Percentile rank
HLA-A*01:01	1	94	107	14	LAEVKALTTELTAE	LAEVKALTE	LAEVKALTTELTAE	7e-06	92
HLA-A*01:01	1	90	103	14	VGKTLAEVKALTTE	VEVKALTTE	VGKTLAEVKALTTE	7e-06	92
HLA-A*01:01	1	48	61	14	VYNGEQQEAIKSAE	VYNGEQQEE	VYNGEQQEAIKSAE	7e-06	92
HLA-A*01:01	1	350	363	14	NVQCTVAEWVLVGQ	NAEWVLVGQ	NVQCTVAEWVLVGQ	6e-06	93

Table 3
HTL: MHC-II Prediction method: IEDB recommended 2.22 | Low adjusted_rank = good binders.

Allele	#	Start	End	Length	Method used	Peptide	Percentile Rank
HLA-DRB1*01:01	1	94	108	15	Consensus (comb.lib./smm/nn)	**LAEVKALTTELTAEN**	1.02
HLA-DRB1*01:01	1	95	109	15	Consensus (comb.lib./smm/nn)	AEVKALTTELTAENQ	2.05
HLA-DRB1*01:01	1	146	160	15	Consensus (comb.lib./smm/nn)	DPLKIKRVHARMAFT	2.10
HLA-DRB1*01:01	1	179	193	15	Consensus (comb.lib./smm/nn)	EKIYGLIAKKQSNLF	3.01

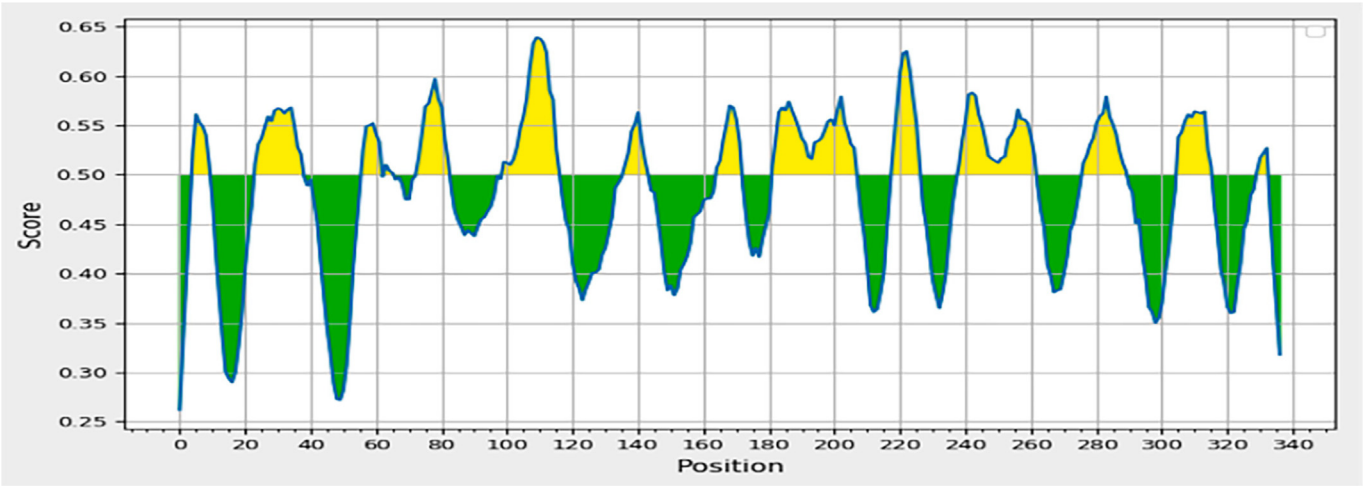

Fig. 5. BCL Epitope Confirmation with BepiPred for 6KMF.

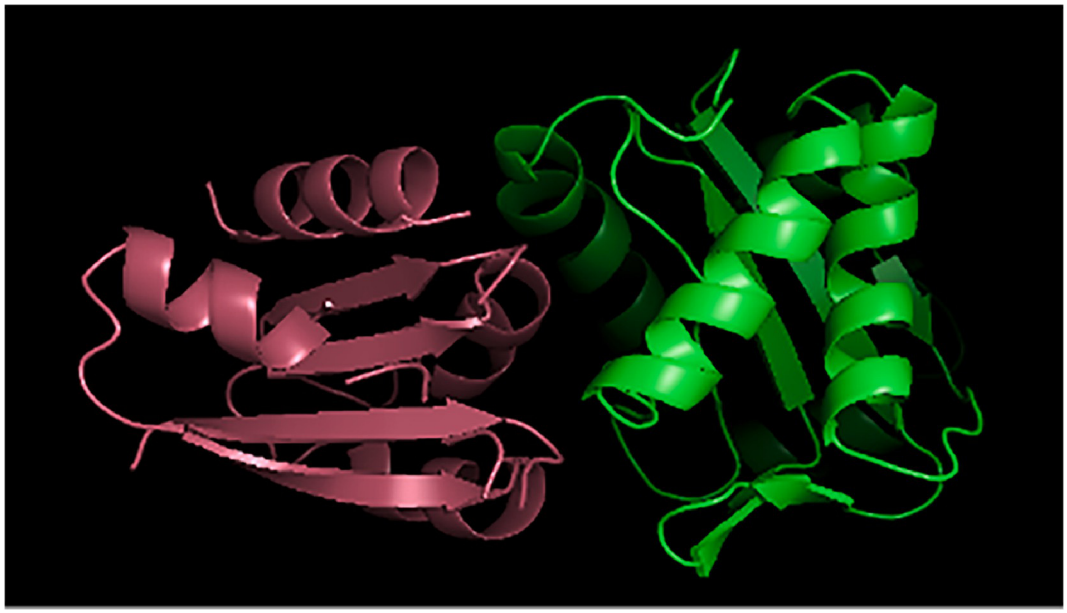

Fig. 6. Molecular Docking of epitope with CXCR4-TLR2.

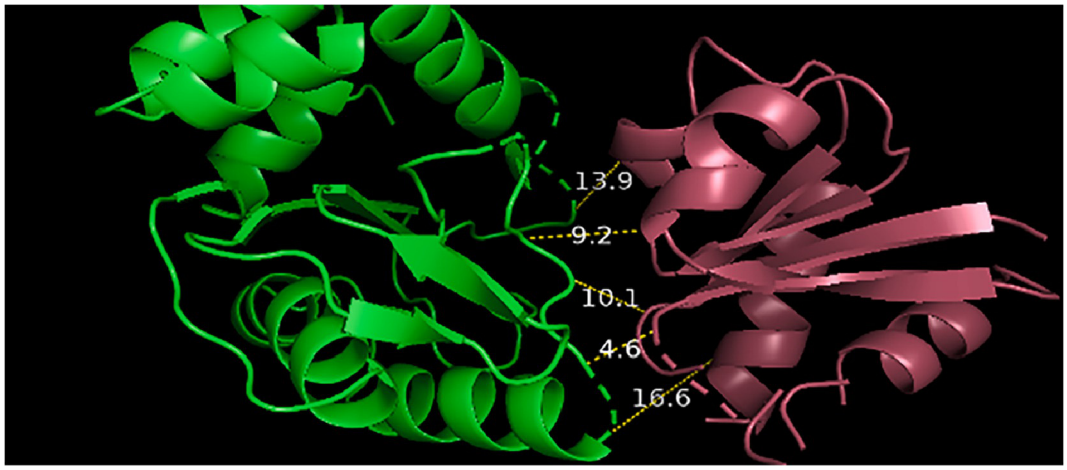

Fig. 7. Molecular docking of epitope with CXCR4-TLR2.

Cluster Scores

We strongly encourage you to read the FAQ related to these scores before using them.

Cluster	Members	Representative	Weighted Score
0	419	Center	-493.2
		Lowest Energy	-595.4
1	124	Center	-454.0
		Lowest Energy	-544.7
2	122	Center	-458.4
		Lowest Energy	-574.7
3	66	Center	-457.1
		Lowest Energy	-586.1
4	29	Center	-459.9
		Lowest Energy	-509.7

Fig. 8. Cluster scores for a vaccine with its center and lower energy.

CXCR4 (TLR2) is an essential immunological receptor for host pathogenesis because it contributes to protective immunity. The fimbriae of *P. gingivalis* comprise polymerized fimbrillin (FimA) and auxiliary proteins (FimCDE) generated by genes in the fimbrial operon. These are a primary colonization component that also contributes to virulence by the immune perversion of TLR signals. Additionally, resistant response generation plays a crucial role in the pathophysiology of *P. gingivalis*, with CXCR4 being precisely targeted to eliminate *P. gingivalis* in *peri*-implantitis (Hajishengallis et al., 2008). As a result, the epitope vaccine was improved by integrating the obtained HTL, CTL, as well as BCL epitopes from *P. gingivalis,* strains 6JKZ & 6KMF. The epitope is then allowed to dock with CXCR4-TLR2 for evaluating a lower binding energy affinity. The molecular docking analysis of the multi-epitope vaccine with the CXCR4(TLR2) immune receptor revealed a higher propensity for interaction, contributing positively to infection-inhibitory activity with the lowest binding energy score of (-595.4 kJ mol $-$ 1). The vaccine's interaction with CXCR4 was more reliable. This may be due to the vaccine's increased affinity for the CXCR4-TLR2 receptor. Further studies with a molecular dynamics simulation design are needed to evaluate the stability of docked protein-peptide complexes.

5. Conclusion

A multi-epitope vaccine incorporating BCL, HTL, and CTL epitopes was constructed for 6JKZ and 6KMF using an immunoinformatics technique. The produced vaccine was non-allergenic and had excellent antigenicity, solubility, and stability. This research demonstrates the vaccine's unique and stable interaction with the CXCR4 (TLR2) immune receptor. It presents with a regular and efficient expression in the bacterial system. As a result, it will evoke a strong memory reaction and mount both cellular and humoral immune responses towards *Porphyromonas gingivalis*. This can significantly reduce *peri*-implantitis and enhance implant stability and survival.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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