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

Pradeep Kumar Yadalam, Santhiya Rengaraj, Maryam H. Mugri, Mohammed Sayed, Amit Porwal, Nasser Mesfer Alahmari, Khaled M. Alzahrani, Ali Robaian, Hosam Ali Baeshen, Shankargouda Patil

Article history:
Received 27 August 2021
Revised 8 September 2021
Accepted 12 September 2021
Available online 17 September 2021

Objective: 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.

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 refer 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.
T. forsythia, an odontal pathogen, causes periodontal disease (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 inflammatory 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 aggravaed 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. actinomycetemcomitans 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, 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 fimbriinlin, 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 lb 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 lb 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 lb fimA was associated with the progressive deepening of the probing depth during the progression of implant disease. The fimA type lb 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 includeRegular 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 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.

CXCR-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-α (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
can help prevent peri-implantitis, bone loss and enhance implant survivability.

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. 6JZK 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/Aller-genFP/) (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. 1. 6JKZ & 6KMF similarity using WATER TOOL software.](image)

![Fig. 2. The antigenic propensity of 6JKZ & 6KMF.](image)
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/) JNN 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.

### Table 1

| ID | Sequence pep | KTSNSNRAF | aff | 0.1388 aff_rescale | 0.5893 cle 0.7978 tap 2.6640 | COMB | 0.8422 < -E |
|----|--------------|-----------|-----|-------------------|-----------------------------|-------|---------------|
| 2  | VAKTVNMY     | aff 0.1095 | aff_rescale | 0.4656 cle 0.5766 tap 3.1380 | COMB | 0.7683 < -E |
| 3  | KAGKNYIGY    | aff 0.1200 | aff_rescale | 0.5096 cle 0.9636 tap 2.9690 | COMB | 0.8026 < -E |
| 4  | MSMAYDNY     | aff 0.7480 | aff_rescale | 3.1739 cle 0.7830 tap 2.9800 | COMB | 3.4423 < -E |
| 5  | YTPITERY     | aff 0.1703 | aff_rescale | 0.7232 cle 0.7824 tap 3.0040 | COMB | 0.9907 < -E |
| 6  | TLVNAADANY   | aff 0.1317 | aff_rescale | 0.5392 cle 0.9420 tap 3.1550 | COMB | 0.8383 < -E |
| 7  | SLTITNGAY    | aff 0.4751 | aff_rescale | 2.0172 cle 0.9263 tap 2.9310 | COMB | 2.3027 < -E |
| 8  | AADAPQGY     | aff 0.5706 | aff_rescale | 2.4226 cle 0.8070 tap 2.8000 | COMB | 2.6837 < -E |
| 9  | YSANGTTIH    | aff 0.1870 | aff_rescale | 0.7939 cle 0.0376 tap -0.6370 | COMB | 0.7677 < -E |
| 10 | WVDARQGTT    | aff 0.5142 | aff_rescale | 2.1832 cle 0.5043 tap 3.0010 | COMB | 2.4089 < -E |
| 11 | LAEVKALTTE   | aff 0.2888 | aff_rescale | 1.2260 cle 0.9734 tap 3.0200 | COMB | 1.5230 < -E |
| 12 | ITESAHLNV    | aff 0.2741 | aff_rescale | 1.1640 cle 0.7765 tap 0.0270 | COMB | 1.2818 < -E |

Fig. 3. The antigenic propensity of 6KMF.

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 lb 6JKZ was aligned with its *P. gingivalis* fimA type lb 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 6JKZ 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 construction (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 were predicted for HLA-DR, HLA-DQ, and HLA-DP loci 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) 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 construction (Table 3).

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 mol\(^{-1}\)) 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 lb 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-allergic. 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 |
|-------------------|----------------|-------|
| TLVNDARVTGSLTT    | 196            | 0.60  |
| LAEVIKALTTELTAENQ | 94             | 0.56  |
| VTEGATISVFTSN     | 12             | 0.55  |
| KLQKNGGADLAGADLAA | 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 | LAEVIKALTTELTAEN | LAEVIKALTTE | LAEVIKALTTELTAEN | 7e-06 | 92 |
| HLA-A*01:01 | 1 | 90 | 103 | 14 | VGKTALAEVKALTTE | VEVKALTTE | VGKTALAEVKALTTE | 7e-06 | 92 |
| HLA-A*01:01 | 1 | 48 | 61 | 14 | VYNGEQAEKSAE | VYNGEQEKE | VYNGEQAEKSAE | 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 |
|--------|--------|-----|--------|-------------|---------|----------------|
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.
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 pervalence 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 was constructed for 6JKZ and 6KMF using an immunoinformatics technique. The produced vaccine was non-allergenic and 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 & 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.

References

Amano, A., 2003. Molecular Interaction of Porphyromonas gingivalis with Host Cells: Implication for the Microbial Pathogenesis of Periodontal Disease. *J Periodontol* 74 (1), 90–96. https://doi.org/10.1902/jop.2003.74.1.90.

Amano, A., Sojar, H.T., Lee, J.Y., Sharma, A., Levine, M.J., Genco, R.J., 1994. Salivary receptors for recombinant fimbrillin of Porphyromonas gingivalis. Infect. Immun. 62 (8), 3372–3380. https://doi.org/10.1128/IAI.62.8.3372-3380.1994.

Botero, J.E., Gonzalez, A.M. Mercado, R.A., Olave, G., Contreras, A., 2005. Subgingival Microbiota in Peri-Implant MucoSA Lesions and Adjacent Teeth in Partially Edentulous Patients. J. Periodontol. 76 (9), 1490–1495. https://doi.org/10.1902/jop.2005.76.9.1490.

de Oliveira, G.R., Pozzer, L., Cavalieri-Pereira, L., de Moraes, P.H., Olate, S., de Albergaria Barbaso, J.R., 2012. Bacterial adhesion and colonization differences between zirconia and titanium implant abutments: An in vivo human study. *J. Period. Impl. Sci. 42*. 217–223. https://doi.org/10.5051/jips.2012.42.6.217.

Enersen, M., Nakano, K., Amano, A., 2013. Porphyromonas gingivalis fimbiae 0–10. 10.1034/j.1600-061x.2012.00730.x.

Enersen, M., Olsen, I., Kvalheim, Ø., Caugant, D.A., 2008. FimA genotypes and multilocus sequence types of Porphyromonas gingivalis from patients with periodontitis. *J. Clin. Microbiol. 46*, 31–42. https://doi.org/10.1128/JCM.00986-07.

Hajishengallis, G., 2011. Immune evasion strategies of Porphyromonas gingivalis. *J Oral Biol* 53, 233–240. https://doi.org/10.1034/j.1601-0822.2011.01075.x.

Hajishengallis, George, Tapping, Richard L, Horakopakis, Evlamyla, Nishiyama, Soichiro, Ratti, Pukar, Schiffler, Robert E., Lyle, Elizabeth A., Triantafylla, Martha, Hamada, Triantafilla, Kathy, Yoshimura, Fuminobu, 2006. Differential interactions of fimbriations and lipopolysaccharide from Porphyromonas gingivalis with the Toll-like receptor 2-centred pattern recognition apparatus. *Cell. Microbiol.* 8 (10), 1557–1570. https://doi.org/10.1111/j.1462-2020.2006.00730.x.

Hajishengallis, G., Wang, M., Liang, S., Triantafylla, M., Triantafylla, K., 2008. Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function. *PNAS* 105 (36), 13532–13537. https://doi.org/10.1073/pnas.0803852105.

Hamada, N., Watanabe, K., Ariai, M., Hikimune, H., Umemoto, T., 2002. Cytokine production induced by a 67-kDa fimbrial protein from Porphyromonas gingivalis. Oral Microbiol. Immunol. 17, 197–200. https://doi.org/10.1111/j.1399-3022.2002.170311.x.

Hishikawa, Jun, Kaisho, Tsumiyasu, Tomizawa, Hitoshi, Lee, Byung Ok, Kobune, Kawabata, Shigetada, Hamada, Shigeyuki, 2002a. Functional differences among FimA variants of Porphyromonas gingivalis fimbrillin: Size, amino-terminal sequence, and antigenic heterogeneity. *Infect. Immun.* 59 (1), 383–389. https://doi.org/10.1128/IAI.70.10.1490-1505.2002.

Microbiota around root-form endosseous implants: A review of the literature, 2003. *J. Prosth. Dent. 89*, 517. https://doi.org/10.1016/s0022-3913(03)00272-5.

Mombelli, A., Lang, N.P., 1998. The diagnosis and treatment of peri-implantitis. *Periodontol 2000* 63–76. https://doi.org/10.1111/j.1462-9992.1998.tb00699.x.

Mombelli, A., Markx, M., Guberti, T., Grunder, U., Lang, N.P., 1995. Clinical periodontitis by the microbiota of osseointegrated implants in patients with a history of periodontal disease. *Cin. Periodontol.* 22, 124–130.

Nagano, K., Abiko, Y., Yoshida, Y., Yoshimura, F., 2013. Genetic and antigenic analyses of Porphyromonas gingivalis FimA fimbrina. *Mol. Oral Microbiol.* 28 (5), 392–403. https://doi.org/10.1111/oom.2013.28.issue-S1110.1110.12032.

Nakagawa, Ikuo, Amano, A, Amano, M., Kubo, Masao, Nakamura, Takayuki, Kawabata, Shigetada, Hamada, Shigeyuki, 2002a. Functional differences among FimA variants of Porphyromonas gingivalis and their effects on adhesion to and invasion of human epithelial cells. *Infect. Immun.* 70 (1), 277–285. https://doi.org/10.1128/IAI.70.1.277-285.2002.

Nakagawa, I., Amano, A., Ohara-Nemoto, Y., Endoh, N., Morisaki, I., Kimura, S., Kawabata, S., Hamada, S., 2002. Identification of a new variant of fimA gene of Porphyromonas gingivalis and its distribution in adults and disabled populations with periodontitis. *J. Periodontol Res.* 37, 425–432. https://doi.org/10.1034/j.1600-0765.2002.01637.x.

Paster, Bruce J., Boches, Susan K., Gavino, Jamie L., Ericson, Rebecca E., Lau, Carol N., Levanos, Valerie A., Sahasrabudhe, Ashish, Dewhirst, Floyd E., 2001. Bacterial diversity in human subgingival plaque. *J. Bacteriol.* 183 (12), 3770–3783. https://doi.org/10.1128/JB.183.12.3770-3783.2001.

Pérez-Chaparro, P.J., Lafuente, G.I., Gracia, P., Meuric, V., Tamanai-Shacoori, Z., Castellanos, J.E., Bonnarme-Mallet, M., 2009. Distribution of porphyromonas gingivalis fimA genotypes in isolates from subgingival plaque and blood sample during bacteremia. *BioMedica* 29, 298–306. https://doi.org/10.7705/biomedica.v29i2.31.

Fig. 8. Cluster scores for a vaccine with its center and lower energy.
Sakka, Salah, Baroudi, Kusai, Nassani, Mohammad Zakaria, 2012. Factors associated with early and late failure of dental implants. J. Invest. Clin. Dent. 3 (4), 258–261. https://doi.org/10.1111/j.2041-1626.2012.00162.x.

Salcetti, J.M., Moriarty, J.D., Cooper, L.F., Smith, F.W., Collins, J.G., Socransky, S.S., Offenbacher, S., 1997. The clinical, microbial, and host response characteristics of the failing implant. Int. J. Oral Maxillofac. Implants. 12 (1), 32–42.

Zhao, L., Wu, Y.F., Yang, H., Meng, S., Ou-Yang, Y.L., 2007. Prevalence of fimA genotypes of Porphyromonas gingivalis and periodontal health status. Hua xi kou qiang yi xue za zhi = Huaxi kouqiang yixue zazhi = West China J. Stomatol. 25, 237–241.

Zitzmann, N.U., Berglundh, T., 2008. Definition and prevalence of peri-implant diseases. J. Clin. Periodontol. 35, 286–291. https://doi.org/10.1111/j.1600-051x.2008.01274.x.