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DNA VACCINE TECHNOLOGY FOR PERIODONTITIS PREVENTION

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ABSTRACT

Periodontitis is a chronic inflammatory disease associated with *Porphyromonas gingivalis* as the main pathogen, characterized by multiple virulence factors such as gingipains, fimbriae, and hemagglutinins. DNA vaccines have been developed as an immunoprophylactic strategy with advantages in safety, stability, and the ability to induce both humoral and cellular immunity. A literature review was conducted using PubMed, ClinicalKey, and Google Scholar (2001–2024) with the keywords "DNA, vaccine" and "periodontitis", screened through PRISMA, resulting in four eligible articles. Results: DNA vaccine candidates included rgpA, kgp, hemagglutinin (HA), and fimbrial protein A (FimA). In animal models, these vaccines enhanced IgG and SIgA production, reduced inflammatory mediators (IL-6, IL-1β, TNF-α), and prevented alveolar bone resorption by up to 60% without organ toxicity. DNA vaccines demonstrate significant potential as preventive immunobiotherapy for periodontitis by effectively targeting *Porphyromonas gingivalis* virulence factors. However, further studies are required on formulation, adjuvant optimization, human clinical trials, and bioethical considerations to ensure clinical translation.

Keywords: DNA vaccine, periodontitis, *Porphyromonas gingivalis*

INTRODUCTION

Vaccines have long been known as substances used to elicit an immune response against pathogenic microorganisms (Friedman et al., 2021). Vaccines stimulate the immune system to produce antibodies that persist long enough to fight antigens from specific pathogens that enter the body (Fujita et al., 2024). DNA vaccines have begun to develop in line with advances in immunology and molecular biology. DNA vaccines are a new approach to vaccination that utilizes one or more genes encoding proteins from a pathogen. This technique has the advantage of inducing cellular or humoral immunity against specific antigens, or both, in a way that conventional vaccines cannot.

Unlike conventional vaccines, which are made using whole viruses or bacteria, or fragments of protein or glucose, DNA vaccines use genetic material from viruses or bacteria (Lukas, 2020). This material provides the body with instructions for producing specific foreign proteins. This teaches the body to recognize these proteins as threats and fight them. Currently, the development of DNA vaccines is still in the research and clinical trials phase.

Direct transfer of plasmid DNA into mouse tissue without a special delivery system was first successfully performed in 1990. Plasmid DNA injected intramuscularly into the mice was able to produce the protein encoded by the DNA sequence contained in the plasmid DNA in the mouse tissue. Subsequent research demonstrated that DNA can be directly inserted in vivo to produce the desired protein according to the DNA sequence that codes for the protein's expression. Since then, it has been believed that the in vivo DNA transfer method can be applied to both gene therapy and DNA vaccination (Bernieri et al., 2020).

DNA vaccines are expected to help reduce the prevalence of various oral and systemic diseases. One oral disease that can worsen systemic conditions is periodontitis. Periodontitis is known to be responsible for increasing the risk of various systemic diseases.

Periodontitis is an inflammation caused by infection of the supporting tissues of the teeth, or periodontal tissues. Genetic, environmental, and behavioral factors are also involved in the development of the disease and accelerate its severity. 1 The prevalence of periodontal disease is reported to range from 20% to 50% worldwide. It is a leading cause of tooth loss, which can affect chewing, aesthetics, self-confidence, and quality of life. 2 According to the 2018 Basic Health Research (Riskesdas), the prevalence of periodontitis in people aged 15 years and older is 67.8%. This means that seven out of ten Indonesians suffer from periodontitis (Aguilera et al., 2020).

Periodontitis commonly occurs in adults and is widespread in the elderly. However, this disease can also affect children and adolescents. Periodontitis has also been linked to systemic conditions, such as diabetes, cardiovascular disease, rheumatoid arthritis, orodigestive cancer, and Alzheimer's disease. This is important to note, given that periodontitis influences the severity of systemic diseases (Preshaw & Bissett, 2019).

Many recent studies have explored the relationship between oral health, inflammation, and systemic disease (Fig. 1). Oral microbiota can not only cause oral inflammation but can also directly contribute to systemic inflammation, increasing inflammation through the release of toxins or the entry of microbial products into the bloodstream (Bobetsis et al., 2023). Understanding the relationship between oral inflammation and systemic inflammation is crucial, particularly given the detrimental impact of oral inflammation on multiple organ systems and the potential for oral disease to increase the risk of developing systemic diseases (Wahyudin & Perceka, 2021). This also makes periodontal pathogens one of the oral diseases that can exacerbate systemic conditions, such as diabetes, cardiovascular disease, rheumatoid arthritis, orodigestive

cancer, and Alzheimer's disease (Aguilera et al., 2020).

Periodontitis is a chronic inflammatory condition of the periodontal tissues, consisting of the gingiva, alveolar bone, periodontal ligament, and cementum. Periodontal disease begins with local inflammation of the gingiva initiated by bacteria in dental plaque. Chronic periodontitis occurs when untreated gingivitis progresses to loss of gingival attachment, alveolar bone, and periodontal ligament, creating the characteristic periodontal pockets that can ultimately lead to tooth loss. Periodontal disease can contribute to systemic inflammation and exacerbate diabetes mellitus and atherosclerosis (Kocher et al., 2018).

Chronic periodontitis is caused by gram-negative bacteria, anaerobes, and microaerophilic bacteria found in the subgingival area, which produce pro-inflammatory prostaglandins and cytokines, leading to periodontal tissue damage. Pathogenic bacteria that cause periodontal disease, such as Porphyromonas gingivalis, can cause inflammation in the periodontal tissues (Nguyen et al., 2020a)

The immune and inflammatory responses that occur are crucial in the development of periodontal disease, damaging tissue and are also influenced by the environment, the patient's genetics, and lifestyle (Munteanu & Schwartz, 2022). The immune system aims to protect the integrity of the individual and prevent the invasion of harmful organisms and substances in the environment that could damage the individual (Falkenberg et al., 2018). It is a coordinated biological response system. Plaque bacteria are the primary cause of periodontal disease. Bacterial products can alter metabolism and inhibit the growth of host tissue. The immune system involves complex interactions between regulatory molecules and cells. Tissue damage is caused by bacterial products. White blood cells, or leukocytes, play a crucial role in the inflammatory response. Leukocytes provide a strong and rapid defense against any infectious agent that may be present. In infections caused by bacteria or other infectious and toxic microbes, an increase in leukocytes may be observed. In acute inflammation, the leukocytes involved are neutrophils and monocytes, while in chronic inflammation, macrophages and lymphocytes play a role (Ruiz-Pozo et al., 2025).

Neutrophils are the initial defense during inflammation, causing an increase in neutrophil numbers. Neutrophils play a crucial role in the defense mechanism against bacterial infections. Neutrophils are the primary phagocytes in the body's defense system against extracellular bacteria. An increase in neutrophils occurs due to inflammation caused by bacterial infection. Polymorphonuclear neutrophils are the first cells to appear in large numbers during the first hour of inflammation. This is due to their high presence in the bloodstream and their high mobility. Furthermore, neutrophils are a key factor in the early stages of an active inflammatory reaction. Through a process called phagocytosis, neutrophils have the ability to actively move like amoebas and ingest various substances. The phagocytosis process is aided by certain opsonin substances that facilitate leukocyte entry and coat the object for digestion.10

The body's defense mechanism against bacteria in dental plaque and in the junctional epithelium and gingival sulcus occurs when neutrophils release lysosomal and granulation enzymes such as lysozyme, elastase, collagenase, and myeloperoxidase during phagocytosis or after necrosis, causing damage to surrounding tissue.

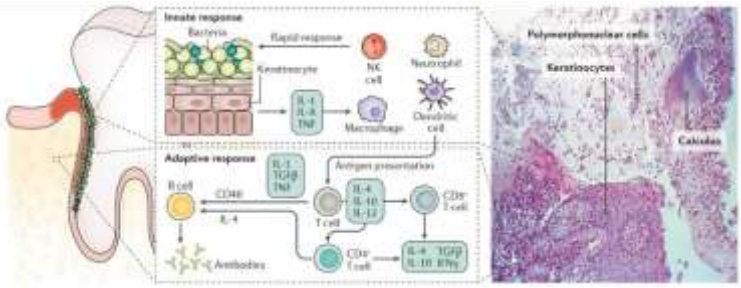

Figure 1. Immune response in chronic periodontitis8

Figure 1 illustrates the interaction between host and pathogen occurring in the gingival crevice and periodontal pocket, characterized by neutrophil and granulocyte (polymorphonuclear cell) infiltration driven by a chemotactic gradient created by bacteria and a lymphocyte inflammatory response followed by antigen presentation by dendritic cells. The resulting pro-inflammatory environment includes cytokines such as tumor necrosis factor (TNF), interleukins, interferon-γ (IFNγ), and transforming growth factor-B (TGFβ), as well as antibodies stimulated by biofilm components. However, neutrophils are eventually overwhelmed by the size and intrusion of the microbial biofilm and are ultimately killed or undergo apoptosis or necrosis upon interaction with bacteria within the gingival crevice.8

Once lymphocytes reach the site of damage, B cells transform into antibody-producing plasma cells. The number and avidity of antibodies are considered crucial in protecting against periodontitis. In addition to antibody responses, T cells contribute to the cell-mediated immune response by stimulating various T helper (TH) cell responses: TH1, TH2, and TH17. TH1 cells may be important during the early stages of chronic periodontitis, while TH2 cells may be relevant in later stages.

However, modern cytokine profiling has revealed that TH9, TH17, TH22, regulatory T cells (Tregs), and other T cell subsets, as well as various cytokines (such as IL-17), are important in the immunopathology of periodontal disease (Nguyen et al., 2020b). An imbalance in these T cell subset responses can contribute to disease and may be related to the function of leukocyte-derived EGF-like repeats and discoidin I-like domain-containing protein 3 (also known as developmentally regulated endothelial cell locus protein 1 (DEL1), an endogenous inhibitor of neutrophil adherence). DEL1 inhibits IL-17-induced oral bone loss in mice, but extrapolating these findings to humans should be done with caution.

Based on this, immunity acquired after vaccination is expected to create resistance to infection. Therefore, periodontitis prevention can be a starting point for systemic disease prevention, one of which can be achieved with DNA vaccines (Dhamodharan et al., 2025). Therefore, further investigation into the potential of DNA vaccine technology for periodontitis prevention is needed. The purpose of this article is to further examine the potential of DNA vaccine technology for periodontitis prevention (Akingbola et al., 2025).

METHODS

The article was written using a literature search through online databases, namely PubMed, ClinicalKey, and Google Scholar. The literature was limited to the years 2001–2024 using the keywords "vaccine," "DNA," and "periodontitis" in English. Articles were processed using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) diagram, as shown in Figure 2. A total of four articles were identified in this literature review.

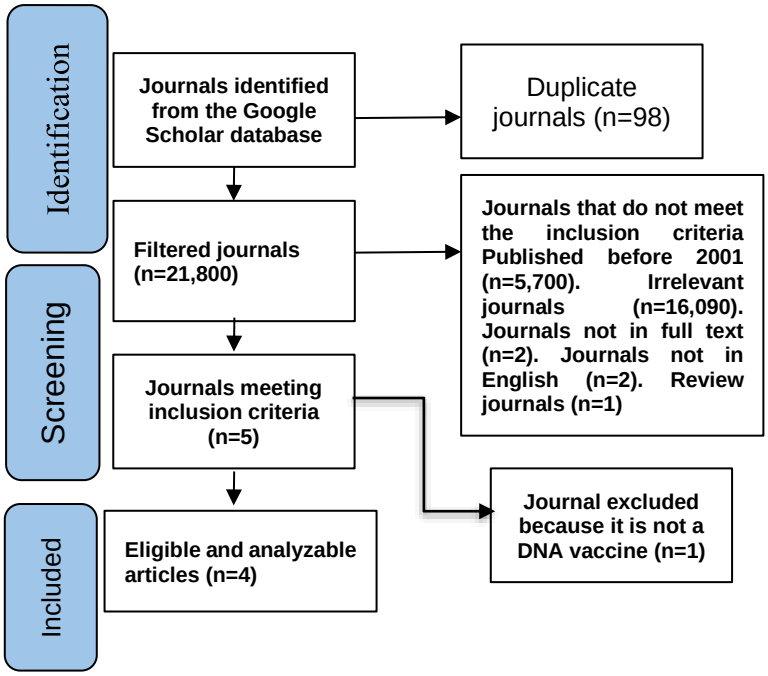

Figure 2. PRISMA Flow

RESULT AND DISCUSSION

1. Result

Tabel 1. Literatur Review Result

Researchers and Years	Vaccine Target	Animal Models	Immunization Methods	Periodontitis Induction	Evaluation	Key Results
Fan et al., 2024	DNA vaccine *rgpA* + domain HA	C57 mice (24 rats)	Intramuscular (3x, 2 week interval)	Molar ligation with thread containing P. Gingivalis	IgG, IgG1, IgG2a, inflammatory cytokines (IL-6, IL-1β, TNF-α), micro-CT, organ histology	IgG increased; inflammatory cytokines and bone loss decreased; no organ toxicity
Zhang et al., 2024	DNA vaccine *HA2-FimA*	Sprague Dawley Mice	Intranasal (3x, 1 week interval)	Molar ligation with P. gingivalis-impregnated suture (4 weeks)	RNA-Seq (gene expression), SIgA, CAMP in saliva, micro-CT, alveolar bone histology	SIgA and CAMP are increased; alveolar bone resorption is reduced; key immune genes are activated.

Jiang et al., 2021	DNA vaccine *kgp*	C57BL/N mice (30 mice)	Intramuscular (3x, 2 week interval)	Molar ligation with thread containing P. gingivalis	IgG, IgG1, IgG2a, inflammatory cytokines (IL-1β, TNF-α, IL-6), micro-CT, histology	IgG increased; inflammation decreased; less alveolar bone loss
Yonezawa et al., 2001	DNA vaccine *rgpA*	BALB/c Mice	Intradermal with Gene Gun	Subcutaneous abscess model with P. gingivalis	ELISA antibody, protease inhibition, collagen adhesion, hemagglutination, abscess	Increases specific antibodies; inhibits protease activity; makes mice more resistant to infection

A vaccine is a biological preparation used to elicit adaptive immunity against a specific infectious disease. Vaccines contain a vaccine agent that resembles a disease-causing microorganism and is often made from a weakened or killed microorganism, its toxins, or one of its surface proteins. The agent in the vaccine stimulates the immune system to recognize the agent as a threat, destroy it, and remember it so that the immune system can recognize and destroy related microorganisms when encountered in the future (Lukas, 2020). Vaccines can be prophylactic (e.g., to prevent or ameliorate the effects of future pathogenic infections) or therapeutic (e.g., a vaccine against cancer). Modern vaccine design is based on the central concept of inducing protective immunity against disease by mimicking the naturally occurring immune response against disease-causing pathogens, but without causing disease. To achieve this, factors determining the interaction between the human organism and the infectious agent must be considered at the population, individual, cellular, and gene levels. Vaccines are developed to protect humans from infectious diseases at a population-based level. This implies that vaccines essentially provide protection for every individual within an immunogenically heterogeneous population.

The adaptive immune response is mediated by antibody-producing B cells (humoral immunity) and by T cells (cellular immunity). Most vaccines provide protection through antibody induction. There is considerable evidence supporting that different types of functional antibodies are important in vaccine-induced protection, which is influenced by immunodeficiency states, studies of passive protection, and immunological data (Drummond et al., 2019).

The concept of DNA vaccines has been tested and applied against a variety of pathogens and tumor antigens. Theoretically and conceptually safe, the non-live vaccine approach is a simple way to induce an immune response. DNA vaccines affect not only humoral immunity but also cellular immunity, targets that are difficult to achieve with traditional vaccines (Fitria, n.d.). DNA vaccines, also called nucleic acid vaccines, are essentially eukaryotic expression plasmids encoding antigen-specific proteins. They have many advantages over traditional vaccines, such as: long-term and stable antigen expression; conformation of the expressed protein, which is similar to the natural protein; and the possibility of creating polyvalent vaccines against multiple pathogens. Specifically, DNA vaccines induce cytotoxic killer T lymphocytes (CTLs),

indicating that a significant shift has occurred in the non-live vaccine platform. The use of DNA also promises to address safety concerns associated with live vaccines, such as those observed in a subset of primates receiving a live attenuated simian immunodeficiency virus (SIV) vaccine, and its potential for transmission to unintended individuals. Furthermore, it avoids the risks associated with the manufacture of killed vaccines, as exemplified by the contamination of polio vaccines with live poliovirus due to manufacturing errors.

The structure and genetic elements of a DNA vaccine consist of two main units: the first is a plasmid propagation unit, which functions to control the replication and multiplication of plasmid DNA in vitro in bacterial cells, according to the desired quantity and volume at the time of production. The second unit consists of a DNA fragment containing the vaccine gene cloned into the plasmid DNA, where this vaccine gene is expected to express the foreign protein in host cells (the human body).DNA vaccine plasmids have a propagation unit that functions for their multiplication in host microbial cells, consisting of DNA fragments for replication and selection markers. In vitro DNA vaccine production typically uses Escherichia coli bacteria. Plasmid DNA is transformed into bacterial cells, then Escherichia coli transformant cells containing plasmid DNA are selected. Escherichia coli clones carrying this plasmid DNA are then cultured in appropriate media on an industrial scale, then the plasmid DNA is isolated, purified, and formulated into a DNA vaccine. After the DNA vaccine is injected into the body, the vaccine synthesis unit will work in the host or human cells. As seen in Figure 3, this vaccine synthesis unit consists of a promoter, introns, signal DNA sequences, vaccine genes encoding proteins or antigens from pathogenic microbes and transcriptional terminators (poly-A), as well as immune stimulatory sequences (ISS) (Kostine et al., 2021). Expression of foreign proteins or antigens in host cells, encoded by the vaccine gene, begins with the promoter and ends with the terminator (poly-A). To increase the potency of DNA vaccines, the DNA plasmid is typically supplemented with an ISS, a hexameric nucleotide that interacts with receptors and enhances the DNA vaccine's immunogenicity.

The mechanism by which DNA vaccines stimulate the immune system is that after the DNA plasmid is injected into the tissue, it replicates autonomously and produces a foreign protein, or antigen, encoded by the vaccine gene. This antigen directly stimulates B cells, which then produce antibodies against the antigen or foreign protein encoded by the DNA plasmid. Cells containing this foreign antigen can then act as antigen-presenting cells, which can then be recognized through specific pathways, either through the Major Histocompatibility Complex (MHC) I pathway on CD8+ T cells or MHC II on CD4+ T cells, thus undergoing different processes to stimulate the body's immune system, as shown in Figure 3. The foreign protein can also directly enter other presenting cells, such as dendritic cells, thus stimulating not only the humoral immune system but also the cellular immune system. Because the process of antigen formation by host cells after DNA vaccination resembles the production of antigens during natural infection with microorganisms, the immune response that occurs as a result of DNA vaccination is the same as the immune response induced by pathogenic microorganisms (Smith et al., 2025).

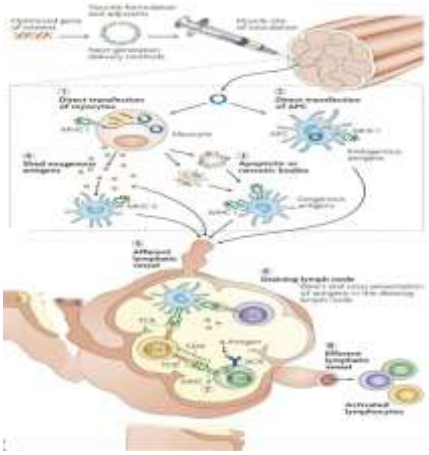

Figure 3. Induction of cellular and humoral immunity by DNA vaccines.

Figure 4 illustrates how the gene sequence of interest (e.g., an antigenic gene or an immune adjuvant) is generated synthetically or by PCR. This sequence is enzymatically inserted into the double-cloning region of the plasmid backbone, purified, and then delivered to the inoculation site by one of several routes of administration: dermal, subcutaneous, or muscular. Through host cellular mechanisms, plasmids enter the nucleus of transfected myocytes (1) and resident antigen-presenting cells (APCs) (2); here, plasmid components initiate gene transcription, followed by protein production in the cytoplasm and the consequent formation of the foreign antigen as a protein or as a peptide sequence. Cells provide endogenous post-translational modifications to the antigen that reproduce the native protein conformation and cells adapt the antigen in a manner similar to that induced by live infection with recombinant vectors. These host-synthesized antigens can then be subjected to immune surveillance in the context of the major histocompatibility class I (MHC I) and MHC II proteins of the vaccinated individual. APCs play a dominant role in the induction of DNA vaccine immunity by presenting vaccine-derived endogenous peptides on MHC I molecules. This can follow either direct transfection by the plasmid vaccine (2) or cross-presentation of cell-associated exogenous antigens; for example, due to APC ingestion of apoptotic transfected cells (3). In addition, APCs mediate peptide display on MHC II molecules after protein antigens secreted from transfected cells are captured and processed in the endocytic pathway (4). Antigen-loaded APCs travel to the draining lymph node (DLN) via afferent lymphatic vessels (5). Presenting peptide antigens to naive T cells via the MHC and T cell receptor (TCR) in combination with co-stimulatory molecules, providing secondary signals necessary for initiating an immune response and T cell expansion (6). In response to peptide-bound MHC molecules and co-stimulatory secondary signals, activated CD4 helper T cells secrete cytokines during cell-to-cell interactions with B cells and bind co-stimulatory molecules necessary for B cell activation (7). In addition, released antigens can be captured by specific high-affinity immunoglobulins (B cell receptors; BCLs) expressed on the surface of B cells in the DLN; they then present the processed antigens to CD4 T helper cells, thereby facilitating the induction of an effective B cell response. In theory, once migratory T cells have been primed in the DLN, they can be re-stimulated and further expanded at the site of immunization by presenting peptide-MHC complexes displayed by transfected muscle cells. These processes coordinately elicit specific immunity against the plasmid-encoded antigen by activating T and B cells, which, now 'armed', can travel through the efferent lymphatic system (8) and provide surveillance. Together, these two arms of the immune system, specifically induced after DNA vaccination, can provide a robust defense against most infectious diseases.

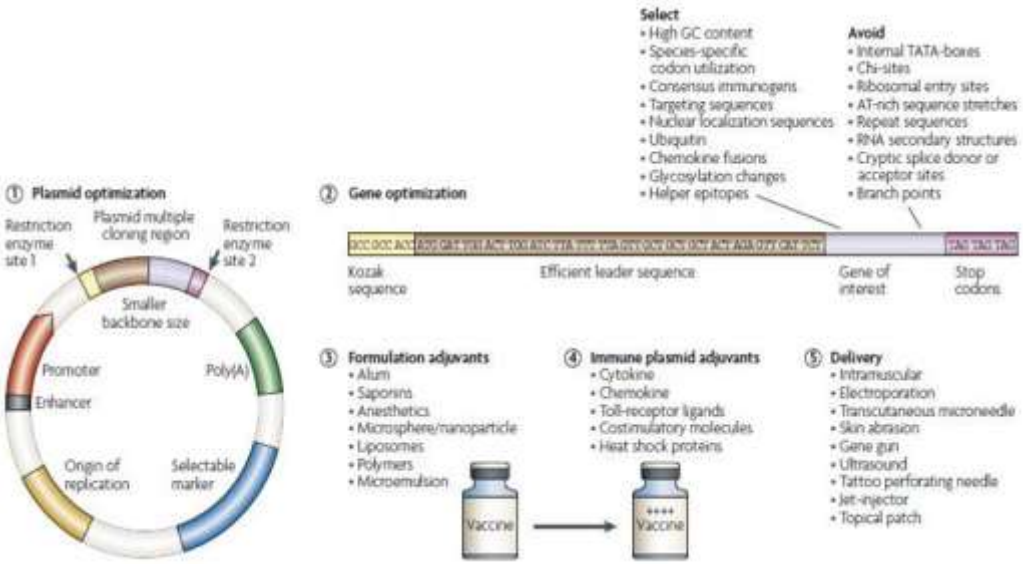

Figure 4. DNA vaccines: optimization strategies to enhance immunogenicity.

Figure 4 shows that DNA vaccine technology has been the target of ongoing efforts to optimize platforms to improve antigen expression and vaccine immunogenicity. Currently, there are several ways antigen expression and immunogenicity can be enhanced for DNA vaccine platforms. These include: optimization of transcription elements on the plasmid backbone (1); strategies to enhance protein expression of the gene of interest (2), including factors to avoid (e.g., chi-sites, which are sequences that promote crossing-over at these sites); the inclusion of formulation aids (3) or immune plasmid adjuvants (4); and the use of subsequent routes of administration (5). Several formulations have been developed and are being tested at all stages of preclinical and clinical development for their ability to enhance antigen expression and immunogenicity (Qi et al., 2020). These mechanisms include encapsulation and protection of DNA from extracellular degradation through particle trapping and high-velocity delivery, with the ultimate goal of delivering the plasmid directly to the cytosol of target cells. Additionally, a class of immune adjuvants encodes immune modulatory molecules targeting death receptors, growth factors, adhesion molecules, cytokines and chemokines, as well as T-receptor ligands. It is noteworthy that many of these plasmid optimization and formulation strategies, as well as adjuvant systems, are used in combination with novel delivery mechanisms, resulting in an overall improved vaccine platform.

Porphyromonas gingivalis, a gram-negative anaerobic bacterium, is the primary etiologic agent of chronic periodontitis. 8 Various virulence factors, including cysteine proteases, lipopolysaccharide (LPS), and fimbriae (FimA), contribute to the pathogenicity of Porphyromonas gingivalis (Katić et al., 2021). Cysteine proteases consist of two arginine residue-specific enzymes, called RgpA and RgpB, and another lysine residue-specific enzyme, called Kgp. These cysteine proteases are thought to play a crucial role in the development of periodontal inflammation through their proteolytic activities, namely, increased vascular permeability through activation of the kallikrein/kinin pathway, dysregulation of plasma clot formation, activation of complement components, and modification of neutrophil function. In Porphyromonas gingivalis infection, it is thought that cysteine proteases and proteases from activated neutrophils synergistically accelerate subgingival tissue destruction through their proteolytic activities. FimA

has been shown to be essential for colonization of mucosal surfaces. Furthermore, it has been shown to have other properties such as the induction of cell adhesion molecules and cytokines from infected cells (Kocak et al., 2023). Although periodontitis in humans and animal models is a polymicrobial disease, immunization with a vaccine containing a single bacterial species, Porphyromonas gingivalis, induced protection. Data indicate that immunization with whole bacterial cells or purified protein preparations considered as vaccine candidates from Porphyromonas gingivalis reduces the rate and severity of bone loss in animal models. It may also temporarily alter the composition of the subgingival microflora (Hummel et al., 2023).

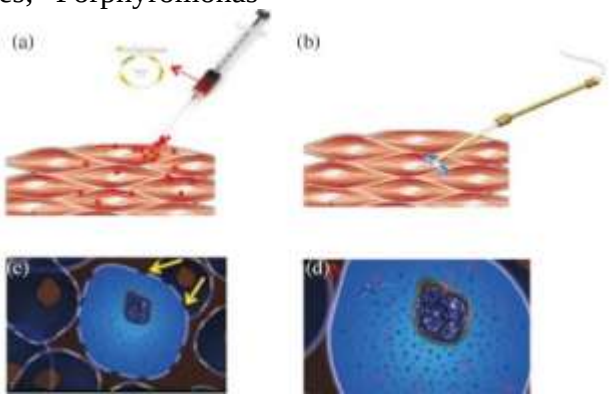

Figure 5. Schematic illustration of electroporation-mediated transfection. a: Intramuscular injection; b: Electroporation; c: Transient increase in cell membrane permeability (yellow arrow) results in plasmid transfer into the cell; d: Cell membrane rupture (red arrow).

Several research reports have demonstrated the effectiveness of DNA vaccines for periodontitis prevention. Porphyromonas gingivalis, one of the bacteria that causes periodontitis, possesses various potential virulence factors, such as hemagglutinin, gingipain, fimbriae, encapsulation, and lipopolysaccharide, which contribute to immune evasion, host immune suppression, and invasion of periodontal tissue. Therefore, several studies have reported the potential for periodontitis prevention with DNA vaccines targeting these virulence factors (Horton & Barrett, 2021). Some studies have focused on the externally secreted virulence factors of Porphyromonas gingivalis, including minor and major fimbriae, Arg-gingipain, and Lys-gingipain antigens (rgpA, kgp), as well as the hemagglutinin (HA) adhesins of rgpA and kgp, with the aim of triggering a protective immune response

(Khan & Jandeleit-Dahm, 2025), have evaluated the potential of an rgpA DNA vaccine in inducing a protective immune response. The rgpA DNA vaccine was tested in mice using the Gene Gun technique. The results showed RgpA mRNA expression in the immunized area and the production of specific antibodies capable of inhibiting the activity of the Rgp proteinase enzyme. Serum from vaccinated mice reduced the ability of Porphyromonas gingivalis to bind to collagen and suppressed hemagglutination activity, which is essential for heme acquisition. In an infection model, immunization with the rgpA DNA vaccine reduced lesion size by 60% compared to controls, indicating significant protection against Porphyromonas gingivalis infection. These findings confirm that rgpA is a potential candidate for a DNA vaccine for periodontitis prevention. Further studies in primates are underway to confirm its effectiveness and clinical feasibility.

The effectiveness of a DNA vaccine based on hemagglutinin-2 (HA2) and fimbrial protein A (FimA) from Porphyromonas gingivalis as a therapeutic strategy for experimental periodontitis in a

mouse model has been studied by Zhang et al. Porphyromonas gingivalis lacks the enzyme for heme synthesis, thus relying on the degradation of host hemoglobin via hemagglutinin-2 (HA2) to obtain porphyrins and trivalent iron essential for its growth and virulence. Therefore, inhibiting the binding between Porphyromonas gingivalis and hemoglobin will reduce the virulence of this bacterium. In addition, fimbriae (FimA) play a crucial role in initial attachment to host tissue and colonization, and also mediate co-aggregation with other oral pathogens such as Treponema denticola, Streptococcus oralis, and Streptococcus gordonii. Both virulence factors (HA2 and FimA) have great potential as candidate vaccine antigens. The trial results showed that immunization of mice with the HA2-FimA DNA vaccine increased mucosal immune responses in the form of SIgA secretion, stimulated the production of antimicrobial peptides (cAMP), and significantly reduced alveolar bone loss in a periodontitis model. The HA2-FimA DNA vaccine was shown to reduce the pathogenicity of Porphyromonas gingivalis through mucosal immune mechanisms and tissue protection. These findings position HA2-FimA as a potential DNA vaccine candidate, which can be further developed as an adjuvant therapy in the prevention and treatment of periodontitis.22

Fan et al.'s research also focused on evaluating the hemagglutinin (HA) and rgpA genes as candidates for DNA vaccine immunization against Porphyromonas gingivalis. The results showed that both genes were able to significantly increase immunoglobulin G (IgG) production and reduce alveolar bone loss due to Porphyromonas gingivalis infection. Specifically, the HA1 domain plays a role in Porphyromonas gingivalis-induced platelet aggregation, while the HA2 domain has a high affinity for hemoglobin and is therefore important for heme acquisition. DNA vaccines expressing HA fragments have been shown to elicit a protective immune response, although their use in genetic vaccines is still rarely studied. This study showed that HA and rgpA DNA vaccines are both immunogenic and immunodominant. In addition, prophylactic immunization reduced levels of key inflammatory mediators, such as IL-6, IL-1β, and TNF-α, in inflamed gingival tissue. The HA DNA vaccine was able to prevent approximately 30% of alveolar bone loss, with effectiveness comparable to the rgpA DNA vaccine. These findings confirm the potential of both genes as candidates for periodontitis preventive vaccines.

In addition, a study by Jiang et al. evaluated the effectiveness of a lysine-gingipain (Kgp)-based DNA vaccine in preventing experimental periodontitis in a mouse model (Belay & Achimano, 2022). The vaccine was constructed using the pVAX1-kgp plasmid carrying a gingipain gene fragment, and the results demonstrated the vaccine's ability to induce an immune response and delay alveolar bone resorption. Previous studies have reported that Kgp has stronger toxic activity and that immunization with Kgp results in more significant alveolar bone protection than RgpA or RgpB, making it a potential target for the prevention and therapy of periodontitis at the genetic level. Structurally, RgpA and Kgp have three important components: a propeptide region (N-terminal), a proteolytic domain, and a homologous hemagglutinin domain (HA, C-terminal). In contrast, RgpB lacks a large HA domain and is therefore thought to be less immunogenic. This explains why RgpA and Kgp are more effective in stimulating adaptive immune responses than RgpB. However, further research is needed to analyze the lectin domain that plays a role in molecular recognition. This domain has the potential to be utilized as a specific immunogen fragment in the development of a more effective vaccine. The pVAX1-kgp DNA vaccine has been shown to stimulate the formation of protective antibodies and reduce alveolar bone damage caused by Porphyromonas gingivalis infection. Based on their immunogenic properties, Kgp, along with RgpA, are promising DNA vaccine candidates for periodontitis prevention strategies. Future development

of more comprehensive and efficient DNA plasmids has the potential to strengthen the effectiveness of protection against periodontal disease. This is supported by the study by Rajapakse et al. that immunization with RgpA-Kgp from Porphyromonas gingivalis limited subgingival crevice colonization by Porphyromonas gingivalis and subsequent periodontal bone loss in a mouse model of periodontitis (Legaki et al., 2022).

Immunobiotherapy is a promising strategy for targeting specific pathogens (Bernieri et al., 2020). Previous research has explored various approaches, including purifying molecules from organisms and using recombinant proteins or molecules, with varying results. One such strategy is DNA vaccines, which are used to prevent various diseases, including periodontitis. DNA vaccines are considered to address challenges faced by conventional vaccines. They are reported to be relatively safe because they only inject genetic information, without live viral particles. They are able to stimulate both humoral and cellular responses (T-helper and cytotoxic), and can be more quickly modified to accommodate mutations or new pathogen variants, with minimal side effects.

Another area that requires further exploration and presents a challenge for researchers going forward is determining which DNA targets are more effective for periodontitis prevention (Vinkers et al., 2021). Adjuvants used in DNA vaccines also reportedly require further study to enhance their effectiveness, especially when testing efficacy in humans. Preclinical animal studies have shown promise, but the immune response in humans is often lower, necessitating specialized formulations. Although considered safe, concerns remain about the potential for DNA integration into the host genome, potentially leading to mutations or cancer, although this risk is very small. However, this study needs to be investigated further (Ballestar et al., 2020).

Furthermore, bioethical challenges must be addressed. Among them, clinical acceptance by doctors and patients may remain skeptical of this new technology, necessitating evidence-based education. Genetic safety will undoubtedly be questioned, raising public concerns about the potential for permanent genetic changes, which, while scientifically small, remains a sensitive issue. Public trust will also be a challenge, given public resistance to DNA-based technology, including the stigma that it is "genetic engineering," which could fuel rejection.

CONCLUSION

DNA vaccines offer advantages such as improved safety, ease of production, storage stability, and the ability to stimulate humoral and cellular immune responses simultaneously compared to conventional vaccines. This review provides strategic insights for the development of DNA vaccines as a preventive approach to periodontitis, particularly against key pathogens such as Porphyromonas gingivalis. Practically, the results of this study open the prospect of clinical trials in at-risk populations and can serve as a basis for integration into the oral immunology research roadmap, thus strengthening the direction of developing immunization-based preventive therapies in medicine.

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