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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

SYSTEMATIC REVIEW

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Periodontal vaccines: a systematic review

Vacinas periodontais: uma revisão sistemática

Swarna MEENAKSHI[1], Sheeja S VARGHESE[2]

1 - Department of Periodontics - Saveetha Dental College - Saveetha Institute of Medical and Technical Sciences - Saveetha University – Chennai - India.
2 - Saveetha Dental College and Hosiptals - Saveetha Institute of Medical and Technical Sciences - Saveetha University - Chennai - India.

ABSTRACT

Background: vaccination is the best known application of immunology to human health. Effective vaccines have successfully eradicated the prevalence of several infectious diseases that were common less than a generation ago. The success of Periodontal vaccines is still elusive due to the complexity of periodontal pathogens that have multiple serotypes. No periodontal vaccine trials have satisfied all the requirements such as preventing colonization of pathogens, protection against tissue destruction and alveolar bone loss, elicit immunoglobulins for phagocytosis, stimulation of T-helper cells. This review aims to discuss the various immunization strategies attempted so far. **Objective:** this review aims to discuss the various in-vitro and in-vivo studies that present supporting evidence for the feasibility of formulating a prophylactic periodontal vaccine that could emerge as an adjunct to mechanical therapy in the future. **Material and Methods:** an extensive literature Search was performed in electronic databases, such as PUBMED, Cochrane central register of controlled trials, Google scholar and science direct using various search terms such as " periodontal vaccines", " porphyromonas gingivalis", "chronic periodontitis", " genomic vaccine ", " recombinant vaccine", "immune response", " vaccination against periodontal bacteria". No limits and language restriction were applied during the electronic search to include all the possible animal studies , clinical trials in the potential relevant article search phase of the systematic review. **Conclusion:** Studies evaluating Porphyromonas gingivalis are the most common and the structures showing the most potential as a vaccine candidate are Outer membrane proteins, fimbriae and gingipains, the structure having the least potential is Lipopolysaccharide.

KEYWORDS

Periodontitis; Vaccine; Virulence; *Porphyromonas gingivalis*; Immune response; Animal studies.

RESUMO

Fundamentação: a vacinação é a aplicação mais conhecida da imunologia à saúde humana. As vacinas eficazes erradicaram com sucesso a prevalência de várias doenças infecciosas que eram comuns há menos de uma geração atrás. O sucesso das vacinas periodontais ainda é ilusório devido à complexidade de patógenos periodontais que possuem múltiplos sorotipos. Nenhum estudo de vacina periodontal atendeu a todos os requisitos, como prevenção da colonização de patógenos, proteção contra destruição de tecidos e perda óssea alveolar, estimulação de imunoglobulinas para fagocitose, estimulação de células T auxiliares. Esta revisão tem como objetivo discutir as várias estratégias de imunização tentadas até o momento. **Objetivo:** esta revisão tem como objetivo discutir os vários estudos in vitro e in vivo que apresentam evidências de apoio à viabilidade de formular uma vacina periodontal profilática que possa emergir como um complemento da terapia mecânica no futuro. **Material e Métodos:** Foi realizada uma extensa pesquisa bibliográfica em bancos de dados eletrônicos, como PUBMED, registro central de ensaios controlados Cochrane, Google Acadêmico e science direct, usando vários termos de pesquisa como "vacinas periodontais", "porphyromonas gingivalis", "periodontite crônica" , "Vacina genômica", "vacina recombinante", "resposta imune", "vacinação contra bactérias periodontais". Nenhum limite e restrição de idioma foi aplicado durante a busca eletrônica para incluir todos os possíveis estudos em animais e ensaios clínicos na fase de busca de artigos potencialmente relevantes da revisão sistemática. **Conclusão:** Estudos avaliando Porphyromonas gingivalis são os mais comuns e as estruturas que mostram maior potencial como candidato a vacina são proteínas de membrana externa, fímbrias e gengivinas, a estrutura com o menor potencial é lipopolissacarídeo.

PALAVRAS-CHAVE

Periodontite; Vacina; Virulência; *Porphyromonas gingivalis*; Resposta imune; Estudos em animais.

INTRODUCTION

Periodontal disease has plagued mankind since the Paleolithic Pre historic era 2.6 million years ago. Bone lesions typical of periodontitis were said to be observed in fossils from the Paleolithic culture of Neanderthal man. There has been descriptions of periodontal diseases in ancient Chinese writings and one of the most common diseases of the Egyptians more than 4000 years ago were said to be a form of suppurating periodontitis [1]. Periodontal diseases have now been studied aggressively for numerous years, and enormous advances have been made in terms of diagnosis, treatment. In spite of the tremendous progress that has been made in treating the disease, many unresolved problems especially the prevention of the disease still remains. Vaccination is one of the best known applications of immunological principles to human health. It has grown in leaps and bounds and has reached a tremendous level since its discovery in the late eighteenth century by Edward Jenner. They stimulate the immune system to produce antibodies without actually infecting the host with the disease. An ideal vaccine should be capable of mimicking the immunological stimulus associated with natural infection, with minimum or no side effects. It should be economical, readily available and easily administered. The recent developments in the fields of immunology, molecular biology and biotechnology has provided various modern tools for proper development of such novel vaccines [2].

There is a plethora of evidences that link periodontitis to various systemic disorders such as atherosclerosis, coronary heart disease ,diabetes mellitus, pre-eclampsia and adverse pregnancy outcomes, osteoporosis, obesity, respiratory disorders and recently polycystic Ovary diseases [3,4]. A successful vaccine for periodontitis could therefore provide health benefits far exceeding the prevention of periodontitis alone. Until the present moment, no preventive modality exists for periodontal disease and treatment rendered is only palliative. The availability of periodontal vaccine would not only prevent or modulate the course of periodontal diseases, but also enhance the quality of life of people for whom periodontal treatment cannot be rendered easily. So far, many animal studies have been done evaluating the role of immunization using potential vaccine candidates, however there is not much focus on details of those vaccine candidates or how efficient they are. This article reviews the various preclinical studies undertaken to develop a potential vaccine and also discusses the possible role of some promising vaccine candidates and future prospects of the same.

Aim of the review

To systematically evaluate the various human and animal studies on vaccination/ immunization against Periodontitis.

Structured question

Is vaccination against periodontitis or periodontopathic pathogens effective?

PICO Analysis

Population: Healthy patients/Animals, Patients with chronic periodontitis, aggressive periodontitis, animals with experimentally induced peiodontitis

Intervention: Immunization or vaccination against periodontal disease or periodontopathic pathogens

Comparison: Non immunized humans / Animals

Outcome: Clinical outcomes such as change or improvement in clinical parameters such as Bleeding on Probing,Gingival Index,Probing pocket depth, Clinical attachment level,Alveolar bone levels. Immunologic outcome such as increased Serum Antibody Titres

Selection Criteria

Inclusion Criteria

Study Design

- Randomized control trials
- Clinical Trials
- Cohort studies
- Animal Experimental studies

Types of Participants

- Human Subjects: Healthy patients,Patients with chronic periodontitis, aggressive periodontitis
- Animal models

Intervention of Interest

- Immunization or vaccination against periodontal disease or periodontopathic pathogens

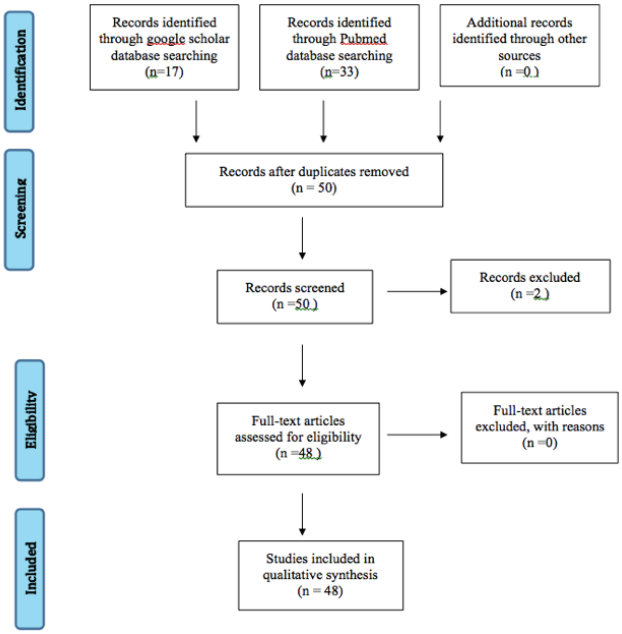
Exclusion Criteria

- Accepted but Unpublished Articles

MATERIAL AND METHODS

A Search was performed in electronic databases, such as PUBMED, Cochrane central register of controlled trials, google scholar, science direct using various search terms such as chronic periodontitis, aggressive periodontitis, experimentally induced periodontal disease, adult periodontitis, adult periodontitis, acute suppurative periodontitis, apical periodontitides,chronic periodontitides, circumpubertal periodontitis, animal model, animal study, preclinical study, monkey study, rat study, dog study, rabbit study, animal studies for periodontal vaccine, periodontal vaccine using non-human primates, periodontal vaccine using murine models, Immunization, vaccination, immune response, genomic vaccine, recombinant vaccine, subunit vaccine, adjuvant, periodontal vaccine, human periodontal vaccine, active immunization, passive immunization, DNA vaccine, acellular vaccine,autogenous vaccine, attenuated vaccine,bacterial vaccine, booster immunization, gene gun DNA immunization. No limits and language restriction were applied during the electronic search to include all the possible clinical trials in the potential relevant article search phase of the systematic review.

PRISMA 2009 Flow Diagram



RESULTS

A total of forty eight studies were included in the present review. (Figure-1) Out of which nine studies were done in Macaca fascicularis models (Table-I) and thirty nine studies were done in Murine models (Table-II).

Forty one immunization studies were done using various components of Porphyromonas gingivalis, four studies were done using Aggregatibacter actinomycetumcomitans and three studies were in other microorganisms (Figure-2).

The studies have evaluated any of the three main parameters such as an increase in serum antibody titer, inhibition of pathogenic microflora and improvement in clinical parameters. (Figure-3) Some studies have evaluated multiple outcome measures as well.

Table I - Studies in macaca fascicularis

S.NO	AUTHORS	ANTIGEN ADMINSTERED (VACCINE)	STUDY TYPE	RESULTS
1.	Niesengard et al. (1989)	Bacteroides macacae (equivalent to P. gingivalis in humans)	Preliminary study in Macaca fascicularis By means of Ligature-induced periodontitis	1. Immunization-induced elevated serum antibody IgG titres to B. macacae 2. The Levels of B. macacae were 2 times higher in non--immunized animals even after 6 months
2.	Ebersole et al. (1990)	leucotoxin vaccine derived from A. actinomycetemcomitans	Animal study to assess serum responses.	Immunization elicited increased IgG responses and increased antibody avidity
3.	Clark et al. (1991)	P. intermedia whole cells	Sub cutaneous, chest,abdomen doses of immunization in 12 monkeys	Increased IgG anti-P.intermedia antibody titres in serum
4.	Persson et al. (1994)	Formalin-killed whole-cell P. gingivalis (5083, primate strain) and Syntex SAF) adjuvant	study in M. fascicularis	Elevated Serum IgG titre to P. gingivalis and presence of Lesser amount of P. gingivalis in immunized animals. Significantly more bone loss in non immunized animals
5.	Persson et al. (1994)	Fimbrial Protein of P.gingivalis	Study in M.fascicularis	A. actinomycetemcomitans, P. gingivalis, P. intermedia, C. rectus, T. forsythia F. nucleatum present in majority of sites tested in non-experimental conditions. Antibody titre levels to P. gingivalis had an inverse correlation with P. gingivalis levels
6.	Moritz et al. (1998)	Purified cysteine protease (porphypain-2) from P. gingivalis versus placebo immunization.	Study of experimental periodontitis in M. fascicularis	Elevated serum IgG titres to whole-cell of P. gingivalis and to Porphypain-2
7.	Roberts et al. (2004)	Formalin killed P.gingivalis strain 5,083 in an SAF adjuvant	10 Macaca fascicularis	IL-1b, TNF-✔ and p gingivalis specific IgG levels were increased in immunized primates
8.	Page et al. (2007)	Cysteine protease of P.gingivalis(150kDa porphpain-1 and 120 kDa posphypain-2) given to experimental group,control group were given buffer with no antigen	Macaca fascicularis (5 control and 5 experimental)	High titres of IgG Antibody in Serum of experimental group
9.	Cox S E et al. (1997)	Cell envelope protein of P.gingivalis, P.intermedia ; Combination of Cell envelope protein of F.nucleatum,A.viscosus,Campylobacter rectus	Macaca Fascicularis	High serum Antibody titres of IgG

Table II - Studies In Murine Models

S.NO	AUTHORS	ANTIGEN ADMINSTERED (VACCINE)	STUDY TYPE	RESULTS
1.	Yamashita et al. (1991)	A.actinomycetumcomitans specific clone A3	30 male Rowett rats	The Serum IgG and IgM Antibody levels were increased after immunization
2.	Evans et al. (1992)	P. gingivalis strains 381, 2561 and P. gingivalis ATCC 33277 fimbriae	Germ-free Sprague–Dawley rats (6 groups of 8 rats per group) Sham-immunized non-infected, Sham-immunized infected, Whole-cell heat-killed P. gingivalis, Purified 43 kDa protein, Purified 75 kDa protein, Combined 43 and 75 kDa vaccine	The rats immunized with purified 43 kDa protein were protected from alveolar bone loss and they had reduced gingival fluid collagenase activity, Purified 75 kDa protein immunized rats had no protection against bone loss, The combination 43, and 75 kDa provided better protection.
3.	Genco et al. (1998)	Extracted cysteine protease (GingipainR) from P. gingivalis A7436 and HG66	148 BALB/c female mice: non-immunized, Peptide A immunized, Gingipain R1 (95 kDa) Gingipain R2 (50 kDa) immunized	When challenged with P. gingivalis, non-immunized and peptide A immunized mice developed ulcerated lesions and pronounced weight loss. Gingipain R immunized mice were protected from abscess formation and had Reduction in viable P. gingivalis counts
4.	Katz et al. (1999)	P.gingivalis ATCC 33277, 381, A7A1–A28, Hagb gene from 381 was cloned in a pET vector	Fischer CD F rats were immunized with recombinant Hagb and Freunds' adjuvant subcutaneously and then orally exposed to fresh P. gingivalis at days 13 and 14 post-immunization	No elevation in salivary IgA in immunized rats, Serum IgG elevated in immunized and infected animals, rHag B immunized rats had a stimulated lymphoid cell culture with high levels of interferon,IL2, IL-1
5.	O'Brien-Simpson et al. (2000)	Rgp– Kgp proteinase adhesion complexof P. gingivalis strain ATCC 33277, P. gingivalis strain W50	BALB/c mice immunized with Rgp–Kgp proteinase adhesion complex of ATCC 33277 or W50 with or without adjuvant (IFA) (Abdominal injections)	Those immunized against W50 showed significantly smaller abdominal lesions when challenged post immunization with W50 When challenged with ATCC 33277, no lesions were found post- immunization.
6.	Gibson III et al. (2001)	Gingipain PgpA and RgpB of P. gingivalis A7A1–A28	BALB/c mice Gingipain PgpA and RgpB, Whole-cell heat-killed P. gingivalis. (3 immunizations)	Immunizations with gingipains stimulates IgG antibodies in serum Immunization with RgpA protects against P. gingivalis-induced alveolar bone loss
7.	Sharma et al. (2001)	Oral immunization with recombinant streptococcus gordonii expressing p gingivals fim A domains	Male Sprague-Dawley germ free rats	Fim-A specific serum IgG and IgA and salivary IgA Antibody responses were increased in immunized rats
8.	Rajapakse et al. (2002)	Whole-cell killed P. gingivalis ATCC 33277/ATCC539781 adjuvant Rgp (ArgX and LysX proteinase) adjuvant	sham immunized Sprague–Dawley rats with 2 immunizations with 3-week intervals	When challenged with P. gingivalis, Immunization with the RgpA–Kgp proteinase induced high IgG antibody titres
9.	Gonzalez et al. (2003)	P.gingivalis capsular protein	6 week old female BALB/c mice	Post Immunization , the Serum IgG and IgM Antibody responses amplified
10.	DeCarlo et al. (2003)	P. gingivalis ATCC 33277 HA2 sequence cloned with E. coli	Fischer CD F rats Test group recombinant HA2 (8 animals) Placebo group (Freunds' adjuvant) (8 animals)	Sham-immunized animals developed no anti-rHA2 IgG2 antibodies, whereas rHA2- immunized animals developed IgG antibodies
11.	Ross et al. (2001)	Recombinant OMP PG32 and PG33	Genetics study with gene vaccine using knockout mice challenged to P. gingivalis infection	Immunization provides reduction in bone lesions
12.	Gemmell et al. (2004)	F. nucleatum ATCC 25586 P. gingivalis ATCC 33277	27 BALB/c female mice. t test group and a placebo group (intra-peritoneal injections once per week for a period of 4 weeks)	Higher levels of IgG1 and IgG2 Antibodies in immunized mice
13.	Satomi Maeba et al. (2004)	Transcutaneos immunization with a 40 kDa OMP of P.gingivalis with Cholera Toxin as an adjuvant	mice	Higher levels of serum IgG and IgA antibodies in Serum, Inhibition of coaggregation of P.gingivalis observed
14.	O'Brien-Simpson et al. (2005)	RgPA-kgP complex and synthetic ABM and proteinase active-side peptides conjugated to diphtheria toxoid (subcutaneous)	BALB/C mice	Mice had P.gingivalis specific IL-4 response , increased IgG Ab, IL-4, IFN-Y response
15.	Lee et al. (2006)	P.gingivalis rHSP60 IFA	12 sprague-Dawley rats	Significantly improved IgG levels post immunization
16.	Hongmei huo et al. (2006)	IL-15 as SIgA-enhancing anti-P. gingivalis Fim A vaccine (Nasal and Intramucosal)	mice	IgG,IgA Ab levels in serum improved
17.	Takahashi et al. (2007)	Fimbriae of P.gingivalis	mice	IgG,IgA Ab levels in serum improved

S.NO	AUTHORS	ANTIGEN ADMINSTERED (VACCINE)	STUDY TYPE	RESULTS
18.	Miyachi et al. (2007)	RgpA DNA vaccine with a gene gun	mice	IgG Ab levels increased
19.	Momoi et al. (2008)	40 kDa OMP of P.gingivalis	Female BALB/c mice	IgG,IgA Ab levels in serum and saliva improved
20.	Kaizumiy et al. (2008)	Transcutaneous immunization with 40 kDa OMP of P.gingivalis without adjuvants	mice	Significant Increase in Serum and Salivary IgA,IgG specific to 40 kDa OMP
21.	Zhang et al. (2009)	40 kDa OMP of P.gingivalis with a DNA vector	Female BALB/c mice	IgG,IgA Ab levels in serum and saliva improved
22.	Liu et al. (2009)	UV inactivated F nucelatum	mice	Decreased Volatile sulphur compounds
23.	Chenlu lui et al. (2010)	Oral Immunization with P.gingivalis 40 kDa OMP and Cpg oligodeoxy-nucleotide	mice	Increased Serum and Salivary IgA,IgG specific to 40 kDa OMP
24.	Yuan Du et al. (2011)	Plasmid PMD157, encoding 25 k-hagA-MBP fusion Protein (Nasal Vaccine)	Experimentally induced periodontitis in mice.	Increased Serum and Salivary IgA,IgG specific to 25k-hag-A-MBP fusion protein
25.	Xiaozhe Han et al. (2014)	Rowett rats were locally injected with whole genomic Pg DNA in alum, Escherichia coli (Ec) genomic DNA, Fuso-bacterium nucleatum and (Fn) genomic DNA . saline/alum injected rats served as controls	Rowette Rats	DNA based adaptive immunity protects host from infection associated with periodontal bone resorption
26.	Neil M O'Brien et al. (2016)	P.gingivalis gingipain Vaccine	Rats	Induced neutralizing IgG1Ab chimera KAS2-A1 Immunogen
27.	Sao Puth et al. (2017)	Mucosal Immunization with a Flagellin-adjuvanated Hgp44 Vaccine	Rats	Intranasal (IN) immunization induced a significantly higher Hgp44-specific IgG titer in the serum of mice than sublingual (SL) administration. The co-administration of flagellin potentiated serum IgG responses for both the IN and SL vaccinations.
28.	Nagasawa et al. (1999)	P.gingivalis Fimbriae in combination with cholera toxin	oral administration in mice	Salivary IgA specific for fimbriae was increased.
29.	Schifferele et al. (1993)	Polysaccharide-BSA conjugate	Administered Intraperitoneally followed by subsequent infection with P.gingivalis	Increased serum Antibody titres evident, Reduced infection after challenge with p.gingivalis
30.	Choi et al. (1998)	Capsule-fimbriae protein conjugate	Administered Intraperitoneally followed by subsequent infection with P.gingivalis	Increased serum Antibody titres of IgG evident, Reduced infection after challenge with p.gingivalis
31.	Chen et al. (1990)	LPS	Murine model	No effect. No evident changes in serum antibody titres
32.	Hamada et al. (2007)	Anti r40 kDa OMP hMAb	Oral administration followed by infection by P.gingivalis	Protection against Bone loss induced by P.gingivalis
33.	Dusek et al. (1994)	Salmonella typhimurium strain x4072 expressing hagB	Intragastric administration	Increased serum and salivary antibody titres of IgA and IgG
34.	Isoda et al. (2007)	S.typhimurium expressing hagB fused to Lpp-OmpA	Oral administration	Increased serum Antibody titres of IgG and IgA
35.	Kawabata et al. (1999)	DNA vaccine: Plasmid PcDNA3/ fimA	Injected into salivary gland	Increased serum fimbriae specific IgA,igG in saliva and serum
36.	Hosogi Y et al. (2001)	Monoclonal Antibody against P.gingivalis hemagglutinin(Mab--Pg-vc)	murine model, hemolytic assay performed	Inhibition of hemolytic activity of P.gingivalis
37.	Zhang et al. (2005)	Recombinant Hemagglutinin domain from Kgp and CTB (B subunit of cholera toxin) compared to other adjuvants	Intranasal delivery to check whether CTB was capable of potentiating the effect of recombinant HA domain of gingipain Kgp	Increased serum Antibody titres in the group that received a combinantion of CTB and recombinant HA domain of Kgp
38.	Takamatsu et al. (1996)	Conjugate Vaccine of A.a serotype b specific polysaccharide antigen and bovine serum albumin	Induced A.a infection in murine model	Rapid healing of lesions were observed and also an increase in serum Antibody titres of IgG
39.	Herminajeng E et al. (2001)	Anti SAM-AA (anti surface associated material of A.actinomycetumcomitans)	Observed immune response in Murine model	Increased serum Antibody titres of IgG

A total of forty studies have evaluated an Increase in Serum Antibody Titres (Table-III), fourteen studies evaluated the inhibition of pathogenic microflora (Table-IV) and twenty studies have evaluated the improvement in clinical parameters (Table-V). Among the forty one studies done in P.gingivalis, three studies have used whole cell as an antigen, four studies have used capsular antigen, seven studies were done on fimbriae, one using lipopolysccharide, seven studies on outer membrane proteins, five studies on hemagglutinins, twelve studies using gingipain, one using heat shock protein and one using the genomic DNA respectively (Figure-4).

Table III - Studies that evaluated the Increase in Serum Antibody Titres

AUTHORS	ANTIGEN ADMINISTERED	ANIMAL MODEL
Niesengard et al. (1989)	Bacteroides macacae	Macaca Fascicularis
Ebersole et al. (1990)	leucotoxin vaccine derived from A. actinomycetemcomitans	Macaca Fascicularis
Persson et al. (1994)	Fimbrial Protein of P.gingivalis	Macaca Fascicularis
Ebersole et al. (1990)	leucotoxin vaccine derived from A. actinomycetemcomitans	Macaca Fascicularis
Clark et al. (1991)	P. intermedia whole cells	Macaca Fascicularis
Persson et al. (1994)	Formalin-killed whole-cell P. gingivalis (5083, primate strain) and Syntex SAF) adjuvant	Macaca Fascicularis
Moritz et al. (1998)	Purified cysteine protease (porphypain-2) from P. gingivalis versus placebo immunization.	Macaca Fascicularis
Yamashita et al. (1991)	A.actinomycetumcomitans specific clone A3	Murine
Katz et al. (1999)	P.gingivalis ATCC 33277, 381, A7A1–A28, Hagb gene from 381 was cloned in a pET vector	Murine
Gibson III et al. (2001)	Gingipain PgpA and RgpB of P. gingivalis A7A1–A28	Murine
Sharma et al. (2001)	Oral immunization with recombinant streptococcus gordonii expressing p gingivals fim A domains	Murine
Rajapakse et al. (2002)	Whole-cell killed P. gingivalis ATCC 33277/ATCC539781 adjuvant Rgp (ArgX and LysX proteinase) adjuvant	Murine
Gonzalez et al. (2003)	P.gingivalis capsular protein	Murine
DeCarlo et al. (2003)	P. gingivalis ATCC 33277 HA2 sequence cloned with E. coli	Murine
Gemmell et al. (2004)	F. nucleatum ATCC 25586 P. gingivalis ATCC 33277	Murine
Satomi Maeba et al. (2004)	Transcutaneos immunization with a 40 kDa OMP of P.gingivalis with Cholera Toxin as an adjuvant	Murine
O'Brien-Simpson et al. (2005)	RgPA-kgP complex and synthetic ABM and proteinase active-side peptides conjugated to diphtheria toxoid (subcutaneous)	Murine
Lee et al. (2006)	P.gingivalis rHSP60 IFA	Murine
Hongmei huo et al. (2006)	IL-15 as SIgA-enhancing anti-P. gingivalis Fim A vaccine (Nasal and Intramucosal)	Murine
Takahashi et al. (2007)	Fimbriae of P.gingivalis	Murine
Miyachi et al. (2007)	RgpA DNA vaccine with a gene gun	Murine
Momoi et al. (2008)	40 kDa OMP of P.gingivalis	Murine
Kaizumiy et al. (2008)	Transcutaneous immunization with 40 kDa OMP of P.gingivalis without adjuvants	Murine
Zhang et al. (2009)	40 kDa OMP of P.gingivalis with a DNA vector	Murine
Chenlu lui et al. (2010)	Oral Immunization with P.gingivalis 40 kDa OMP and Cpg oligodeoxynucleotide	Murine
Yuan Du et al. (2011)	Plasmid PMD157, encoding 25 k-hagA-MBP fusion Protein (Nasal Vaccine)	Murine
Xiaozhe Han et al. (2014)	Rowett rats were locally injected with whole genomic Pg DNA in alum, Escherichia coli (Ec) genomic DNA, Fusobacterium nucleatum and (Fn) genomic DNA . saline/alum injected rats served as controls	Murine
Neil M O'Brien et al. (2016)	P.gingivalis gingipain Vaccine	Murine
Sao Puth et al. (2017)	Mucosal Immunization with a Flagellin-adjuvanated Hgp44 Vaccine	Murine
Persson et al. (1994)	Formalin-killed whole-cell P. gingivalis (5083, primate strain) and Syntex SAF) adjuvant	Macaca Fascicularis
Page et al. (2007)	Cysteine protease of P.gingivalis(150kDa porphpain-1 and 120 kDa posphypain-2) given to experimental group,control group were given buffer with no antigen	Macaca Fascicularis
Nagasawa et al. (1999)	P.gingivalis Fimbriae in combination with cholera toxin	Murine
Schifferele et al. (1993)	Polysaccharide-BSA conjugate	Murine
Choi et al. (1998)	Capsule-fimbriae protein conjugate	Murine
Chen et al. (1990)	LPS	Murine
Dusek et al. (1994)	Salmonella typhimurium strain x4072 expressing hagB	Murine
Isoda et al. (2007)	S.typhimurium expressing hagB fused to Lpp-OmpA	Murine
Kawabata et al. (1999)	DNA vaccine: Plasmid PcDNA3/fimA	Murine
Zhang et al. (2005)	Recombinant Hemagglutinin domain from Kgp and CTB (B subunit of cholera toxin) compared to other adjuvants	Murine
Takamatsu et al. (1996)	Conjugate Vaccine of A.a serotype b specific polysaccharide antigen and bovine serum albumin	Murine
Herminajeng E. et al. (2001)	Anti SAM-AA (anti surface associated material of A.actinomycetumcomitans)	Murine
Cox S E et al. (1997)	Cell envelope protein of P.gingivalis, P.intermedia ; Combination of Cell envelope protein of F.nucleatum,A.viscosus,Campylobacter rectus	Macaca Fascicularis

Table IV - Studies that evaluated the Inhibition of Pathogenic Microflora

AUTHOR	ANTIGEN ADMINISTERED	ANIMAL MODEL
Niesengard et al. (1989)	Bacteroides macacae (equivalent to P. gingivalis in humans)	Macaca Fascicularis
Clark et al. (1991)	P. intermedia whole cells	Macaca Fascicularis
Liu et al. (2009)	UV inactivated F nucelatum	Murine
Persson et al. (1994)	Formalin-killed whole-cell P. gingivalis (5083, primate strain) and Syntex SAF) adjuvant	Macaca Fascicularis
Persson et al. (1994)	Fimbrial Protein of P.gingivalis	Macaca Fascicularis
Page et al. (2007)	Cysteine protease of P.gingivalis (150kDa porphpain-1 and 120 kDa posphypain-2) given to experimental group,control group were given buffer with no antigen	Macaca Fascicularis
Genco et al. (1998)	Extracted cysteine protease (GingipainR) from P. gingivalis A7436 and HG66	Murine
Gibson III et al. (2001)	Gingipain PgpA and RgpB of P. gingivalis A7A1–A28	Murine
Sharma et al. (2001)	Oral immunization with recombinant streptococcus gordonii expressing p gingivals fim A domains	Murine
Satomi Maeba et al. (2004)	Transcutaneos immunization with a 40 kDa OMP of P.gingivalis with Cholera Toxin as an adjuvant	Murine
Momoi et al. (2008)	40 kDa OMP of P.gingivalis	Murine
Zhang et al. (2009)	40 kDa OMP of P.gingivalis with a DNA vector	Murine
Xiaozhe Han et al. (2014)	Rowett rats were locally injected with whole genomic Pg DNA in alum, Escherichia coli (Ec) genomic DNA, Fusobacterium nucleatum and (Fn) genomic DNA . saline/alum injected rats served as controls	Murine
Hosogi Y et al. (2001)	Monoclonal Antibody against P.gingivalis hemagglutinin(Mab-Pg-vc)	Murine

Table V - Studies that evaluated the Improvement in Clinical Parameters

AUTHOR	ANTIGEN ADMINISTERED	ANIMAL MODEL
Roberts et al. (2004)	Formalin killed P.gingivalis strain 5,083 in an SAF adjuvant	Macaca Fascicularis
Yamashita et al. (1991)	A.actinomycetumcomitans specific clone A3	Murine
Evans et al. (1992)	P. gingivalis strains 381, 2561 and P. gingivalis ATCC 33277 fimbriae	Murine
O'Brien-Simpson et al. (2000)	Rgp– Kgp proteinase adhesion complexof P. gingivalis strain ATCC 33277, P. gingivalis strain W50	Murine
Gibson III et al. (2001)	Gingipain PgpA and RgpB of P. gingivalis A7A1–A28	Murine
Gonzalez et al. (2003)	P.gingivalis capsular protein	Murine
DeCarlo et al. (2003)	P. gingivalis ATCC 33277 HA2 sequence cloned with E. coli	Murine
Ross et al. (2004)	P. gingivalis strains YH522, ATCC 33277, ATCC53978	Murine
Lee et al. (2006)	P.gingivalis rHSP60 IFA	Murine
Takahashi et al. (2007)	Fimbriae of P.gingivalis	Murine
Zhang et al. (2009)	40 kDa OMP of P.gingivalis with a DNA vector	Murine
Yuan Du et al. (2011)	Plasmid PMD157, encoding 25 k-hagA-MBP fusion Protein (Nasal Vaccine)	Murine
Genco et al. (1998)	Extracted cysteine protease (GingipainR) from P. gingivalis A7436 and HG66	Murine
Momoi et al. (2008)	40 kDa OMP of P.gingivalis	Murine
Xiaozhe Han et al. (2014)	Rowett rats were locally injected with whole genomic Pg DNA in alum, Escherichia coli (Ec) genomic DNA, Fusobacterium nucleatum and (Fn) genomic DNA . saline/alum injected rats served as controls	Murine
Schifferele et al. (1993)	Polysaccharide-BSA conjugate	Murine
Choi et al. (1998)	Capsule-fimbriae protein conjugate	Murine
Hamada et al. (2007)	Anti r40 kDa OMP hMAb	Murine
Takamatsu et al. (1996)	Conjugate Vaccine of A.a serotype b specific polysaccharide antigen and bovine serum albumin	Murine

Porphyromonas Gingivalis

Capsule

The capsular polysaccharide is a well known virulence factor of P.gingivalis .However, the number of studies carried out in this aspect are only a handful.

A study by Choi et al. in a murine model, immunization with a conjugate vaccine consisting of Porphyromonas gingivalis capsular polysaccharide and Fimbriae led to an increased serum antibody titre of Immunoglobulin G.[5] However, it was not known whether capsule of Porphyromonas gingivalis was capable of eliciting the immune response on its own as P. gingivalis Fimbriae was also a part of the conjugate vaccine administered. Later on, Gonzalez et al. in his study went on to demonstrate that capsule as such was capable of eliciting an increase in titers of IgG and IgM and also provided protection against P.gingivalis induced alveolar bone loss [6].

Fimbriae

Electron microscopic studies of Porphyromonas gingivalis have shown that it possesses three types of fimbriae [7].They consist of major and minor fimbriae and both are said to make a significant contribution to the virulent nature of porphyromonas gingivalis. However, most of the studies have focused on major fimbriae.

In a study by Evans et al. in rats, it was observed that subcutaneous injection with a highly purified 43 k-Da fimbrial protein elicited Fim A-specific antibodies in serum and saliva that conferred protection against P.gingivalis induced alveolar bone loss [8].

Takahashi et al. administered P.gingivalis fimbrial antigen ATCC 33277 with recombinant cholera toxin B subunit in BALB/c mice intranasal and observed a significant immune response [9]. In addition to intranasal and intramuscular immunization, Kawabata et al. presented that targeted salivary gland immunization by injecting a fimA DNA vaccine into the salivary gland using plasmid pcDNA3/fimA showed an enhanced serum and salivary IgG [10].

Lipopolysaccharides

Based on the studies by Chen et al. [11] Elkins et al. [12]. LPS as a vaccine did not show any induction of serum Antibody Titres or protection against P.gingivalis induced alveolar bone loss. Therefore, LPS based vaccines may not confer any potential benefits as a vaccine candidate.

Outer membrane proteins

The various outer membrane proteins identified in P.gingivalis are OmpA-like proteins Pgm 6 (40 kDa) ,Pgm 7(120 kDa), PG 32/33 (43 and 42 kDa) , OmpA40-41 (40 and 41 kDa), RagA and RagB proteins, TonB-dependent OMPs.

Among these OMPs, only the OmpA-like protein PG32/33 and the 40 kDa OMP have been extensively investigated with respect to immunization against P.gingivalis. In a study by Ross et al. [13], subcutaneous immunization with recombinant PG32 and PG33 cloned for expression in E.Coli, significantly reduced the lesion size in mice infected with P.gingivalis. In a study by Hamada et al, oral immunization with 40-kDa OMP showed protection against oral P.gingivalis induced alveolar bone loss in rats.[14] Maeba et al. in their study proved that nasal immunization of mice with 40 kDa OMP and CT induced serum IgG, IgA antibodies and also IgG in saliva [15].

Hemagglutinin

Hemagglutinins as the name suggests are capable of agglutinating erythrocytes thereby adhering to host tissues and playing a major role in virulence of P.gingivalis [16]. The hemagglutinins reported are Hag A,HagB,C,D and E of which HagB has been studied as a vaccine candidate against P.gingivalis.In a study by Katz et al. subcutaneous immunization of rats with recombinant HagB derived from P.gingivalis 381 showed an enhanced serum IgG response [17].

In a study by Zhang et al. in 2005 recombinant Hemagglutinin/adhesion domain from the gingipain K(termed Kgp-rHArep) and the B subunit of Cholera Toxin(CTB) adjuvant delivered nasally were studied in murine model [18]. They found that CTB adjuvant potentiated the immunogenicity of Kgp-rhArep. Furthermore, anti-Kgp-rHArep antibodies were shown to protect against P.gingivalis invasion of epithelial cells invitro.

In a subsequent study by Yuan Du et al. in 2011, they found that Nasal Immunization with a Fusion Protein Consisting of the Hemagglutinin A Antigenic Region and the Maltose-Binding Protein was capable of eliciting CD11c,CD8 Dendritic Cells for Long-Term Protective Immunity [19].

Gingipains

Gingipains is used to describe cysteine proteases. They are grouped into two based on the substrate they cleave into RgpA and RgpB. RgpA cleaves proteins at arginine residue and gingipain K cleaves protein at lysine residue. The catalytic domains present in these gingipains are capable of evading host defense by degrading immunoglobulins, altering function of neutrophils and degrading complement proteins. In addition to this, they degrade C3 derived opsonin thereby rendering P.gingivalis resistant to phagocytosis [20]. Thus a vaccine targeting this component may provide protection against both invasive and noninvasive strains of Porphyromonas gingivalis.

A study by Moritz et al. in Macaca fascicularis demonstrated that active immunization with a purified P.gingivalis cysteine protease elicited significantly heightened IgG antibody titres in the serum [21]. Page et al. found that subcutaneous immunization of Macaca fascicularis with purified cysteine protease from P.gingivalis resulted in a high induction of serum IgG titres and reduction of P.gingivalis in subgingival plaque and reduced alveolar bone loss [22]. Neil O'Brien et al. found that gingipain vaccine was able to induce a serum antibody response as well as resulted in reduced alveolar bone loss in rats [23].

Other studies by Han et al. [24], Sao Puth et al. [25] on whole genomic DNA vaccines and Flagellin vaccines have also showed promising results in inducing serum IgG titres, reduction in alveolar bone loss in experimental animals.

Aggregatibacter Actinomycetumcomitans

After P.gingivalis, Aggregatibacter actinomycetumcomitans is considered an important pathogen in progression of periodontal disease. Takamatsu et al. in their study found that subcutaneous and intranasal immunization of mice with capsular serotype b specific polysaccharide antigen produced a specific antibody which efficiently opsonized Aggregatibacter actinomycetumcomitans serotype b [26]. However, very few studies have been conducted on development of a vaccine targeting Aggregatibacter actinomycetumcomitans.

Other microorganism

Cox et al. in their study in Macaca fascicularis ligature induced periodontitis model reported that parenteral immunization with cell envelope antigen (CEA) of either P.gingivalis, [27] Prevotella intermedia or a combination of cell wall antigens with CEA of Campylobacter rectus,F.nucleatum and Actinomyces viscosus lead to a significant increase in serum IgG, IgM and IgA antibody levels.

Transgenic Microorganisms

A Study by Sharma et al. in mice reported that oral coimmunization of germ-free rats with two S.gordonii recombinants expressing epitopes of P.gingivalis FimA-induced FimA-specific serum (IgG and IgA) and salivary IgA antibody responses.This gave protection against bone loss induced by P.gingivalis [28].

Human Studies

There are no human clinical trials until now. Longitudinal studies of human periodontal diseases present limitations in assessing the mechanisms of disease because there are numerous variables that are difficult to control among patients, including activity level, susceptibility, progression, and duration of the disease [29]. Aukhil et al. (1988), Kohyama et al. (1989) and Sjostrom et al. (1994) in their longitudinal studies measured the reduction in serum IgG titers to different periodontal pathogens like *P. gingivalis, T. denticola, A. actinomycetemcomitans* and *F. nucleatum* following initial periodontal therapy [30,32].

Craig et al. in 2002, measured the serum IgG antibody response to six periodontal pathogens among three different population groups and found a positive correlation [33].

DISCUSSION

Based on the results obtained from this systematic review, the organism which has been investigated the most in formulating a periodontal vaccine is Porphyromonas gingivalis, the least promising vaccine candidate could be the lipopolysaccharide. Studies on LPS did not show induction of any antibody response or significant clinical or microbiological improvements. The most promising vaccine candidates could be outer membrane proteins, fimbriae and gingipains. Individually each of the components were able to stimulate a heightened immune response. A candidate vaccine combining these factors could prove effective against Porphyromonas gingivalis mediated destruction. One more observation was that, even though studies on capsule have shown promising results ,they were among the least investigated components so far.

Until now, the development of Vaccines for Human usage has always relied on conventional approaches that are time consuming and requires the pathogen to be cultured in laboratory conditions and could identify only the abundant antigens. However, with the rapid advancements in bioinformatics, it is possible to study the complete genome sequence of an organism. Vaccine candidates can be identified from this method called " reverse vaccinology" [34,35]. This approach was first used to develop a vaccine against serogroup B meningococcus by identifying the antigens that could be used as potential candidates [36]. This approach has been tried against Porphyromonas gingivalis as well. From anticipated 2000 genes, 120 proteins were selected using Bioinformatics tools for and 107 of these were expressed in E.coli and further analyzed by western blotting method using sera from patients suffering from periodontitis and animal antisera [37]. These 107 candidates were reduced to 40 proteins, which were then purified and used to immunize mice. Following Immunization, the mice were then challenged with the bacteria. From this subset of 40 candidates, two antigens demonstrated highly significant protection in the animal model. These two antigens have shown homology to OprF of Pseudomonas aeruginosa, which is part of the vaccine formulation which is currently used in human clinical trial which is the first of its kind in the world. [38] Very recently, Huang et al. have used a novel technique of cell free protein synthesis as a platform to produce vaccine candidates. They were able to recombinantly generate a cocktail of P.gingivalis proteins that were capable of eliciting a high serum IgG response as well as were capable of protecting the mice from progressive bone loss [39].

CONCLUSION

So far, major efforts have been dedicated to the development of vaccines for highly prevalent diseases. However, with the findings of associations between periodontal disease and other systemic diseases, the contemplation whether there was any merit in developing a vaccine against periodontitis was put to rest and research accelerated in this direction with the focus

being shifted to prevention of the disease. There are numerous studies in animal models that have yielded good results in terms of improvements in serum Antibody titres, reduction in bone loss. However, none have been able to identify a sole or complete vaccine candidate suitable for human usage yet. A candidate vaccine combining various components of the microbe could be one of the best solutions that could prevent periodontal disease as such.

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S.Swarna Meenakshi
(Corresponding address)
Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
E-mail: swarna.meenakshi@gmail.com

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