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The anti-caries vaccine

A systematic integrative review

Anatole Simon Alfred Hippolyte GRAZIANI

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob a Orientação da Professora Doutora Carla Maria Carvalho Batista Pinto

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RESUMO

Introdução: As cáries dentárias são uma doença multifatorial, que implica interações entre os fatores do hospedeiro e o biofilme. A OMS considera a cárie um grave problema de saúde nos países industrializados.

Objetivo: Discutir sobre as novas formas promissoras de construção de uma vacina anti-cárie, os alvos potenciais e as limitações que poderia ter.

Material e métodos: A pesquisa foi realizada no PubMed e Science direct. A pesquisa complementar foi realizada através das bibliografias de outras revisões sistemáticas e trabalhos de meta-análise. Toda a inclusão de estudos foi feita entre Janeiro e Maio de 2022.

Resultados: 29 artigos foram incluídos.

Discussão: As vacinas de ADN, mucosas e subunidades são alguns dos tipos de vacinas mais promissores. Novos adjuvantes, como a combinação de chitosano + Pam3CSK4 ou chitosano + MPL, proteína FliC, ou uma coadministração de CCL19 com CCL17, têm sido testados. Todos eles mostraram boas perspectivas para o futuro. Sistemas de distribuição como nanopartículas ou sprays intranasais tiveram bons resultados. O alvo da vacina que teve as melhores perspectivas de sucesso foi o *S. mutans*, cuja agregação pode ser impedida.

Conclusão: As cáries podem afetar qualquer pessoa em qualquer idade, causando dor e desconforto, diminuindo as interações sociais e a produtividade no trabalho. A cárie é um fator de desigualdade social: as classes sociais mais baixas são mais afetadas. Uma vacina anti-cárie poderia vir com muitas limitações, como o tempo necessário para a desenvolver, a resistência da população à vacinação, os custos a longo prazo, e a possibilidade de diminuir a carga de trabalho para a profissão de dentista.

Palavras-chave: vacina, imunização, cárie dentária, economia, *Streptococcus mutans*, vacina de ADN

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ABSTRACT

Introduction: Dental caries is a multifactorial disease, which involves interaction between host factors and the biofilm. WHO considers caries a major health problem in most industrialized countries.

Objective: To discuss about the new promising ways of making an anti-caries vaccine, the potential targets, and the limitations it could have.

Material and methods: A search was performed in PubMed and Science direct databases. Complementary research was performed through the bibliographies of other systematic reviews and meta-analysis works. The whole inclusion of studies was done between January and May 2022.

Results: 29 articles were included.

Discussion: DNA, mucosal and subunit vaccines are some of the most promising types of vaccine. New adjuvants, like the combination of chitosan + Pam3CSK4 or chitosan + MPL, FliC protein, or a coadministration of CCL19 with CCL17, are being tested. All of them showed good perspectives for the future. Delivery systems like nanoparticles or intranasal sprays had good results. The vaccine's target that had the best prospects of success was *S. mutans*, whose aggregation could be prevented.

Conclusion: The caries can affect anyone at any age, causing pain and discomfort, decreasing social interaction and productivity at work. The caries is a factor of social inequality: lower social classes are more impacted. An anti-caries vaccine could come with many limitations, like the time needed to develop it, the adversity for vaccination among the population, the long-term costs, and the possibility of decreasing the workload for the dentistry profession.

Key words: vaccine, immunization, dental caries, economy, *Streptococcus mutans*, DNA vaccine

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INDEX OF ABREVIATIONS

AgNPs: silver nanoparticles

NP: Nanoparticle

APC: antigen-presenting cell

DC: dendritic cell

DMBT1: Deleted in malignant brain tumors 1

ECC: Early childhood caries

ECGC: Epigallocatechin gallate phospholipid complex

e.g : for example

Fabs: antigen-binding fragments

GTF: glycosyltransferases

GTB: glucan-binding protein

I.e: that Is

LTCD4: T lymphocyte cluster of differentiation 4

MHC: major histocompatibility complex

miR-9: microRNA-9

MPL: monophosphoryl lipid

MS: mutans streptococci

S.: Streptococcus

S-ECC: Severe-early childhood caries

VLP: virus-like particles

WHO: World Health Organization

1. INTRODUCTION

Dental caries is a multifactorial disease, which involves interaction between host factors (tooth surface, acquired pellicle, saliva, diet sugar) and the biofilm. The acid produced by the bacteria of the biofilm will demineralize the enamel, then continue to the dentin to reach the pulp, causing pain and discomfort.(1) At the age of 6 weeks, the oral microbiome of babies is mainly streptococci and *Veillonella* species. Eruption of teeth, puberty and pregnancy, such as other environmental changes, will affect the oral microbiome, promoting fitness and survival (2).

From Jenner and his first vaccine on the XVIII century, vaccines are considered one of the greatest public health achievements. They are an antigenic preparation, which causes an active immune response to prevent or decrease a disease. There are two main types of vaccines: the live attenuated vaccine, which contains the weakened virus; and the non-live vaccine, which doesn't have any virus still alive in it. Today, new types of vaccines, such as the DNA vaccine, are currently studied (3,4).

Nowadays, dental caries is still a neglected topic. WHO considers it a major health problem in most industrialized countries, in which 60-90% of children and most adults are affected (1). From the ongoing Global Burden of Disease Study, it's affecting around 3.9 billion people worldwide, making it a high prevalent disease (1). Plus, untreated caries in permanent teeth is the most prevalent condition evaluated across all medical condition, with a global prevalence of 35% for all age combined with 2,4 billion people affected (1).

During the last 40 years, the anti-caries vaccine is being developed over a large number of clinical trials. If this is successful, it could totally revolution the modern dentistry.

The aim of this work is to provide an overview of the most recent studies about anti-caries vaccines and all that underlies it.

2. OBJECTIVE

To discuss about the new promising ways of the anti-caries vaccine, the potential targets, and the limitations it could have.

3. MATERIAL AND METHODS

This work is an integrative systematic review.

The search was conducted on PubMed and Science Direct.

I used the words « vaccines » and « caries » as MeSH terms and, as key words, "economic", "DNA vaccine", "immunization", "*Streptococcus*", "dental caries", "dental disease", "fluoridation" and "prevention". I excluded some key words like "covid-19" to make the search more pertinent.

The criteria of inclusion were: the language (English and French); articles from 2012 to 2022; and articles that are clinical trial.

For exclusion criteria, I considered articles that came twice or more, articles published before 2012, and articles not related with the subject.

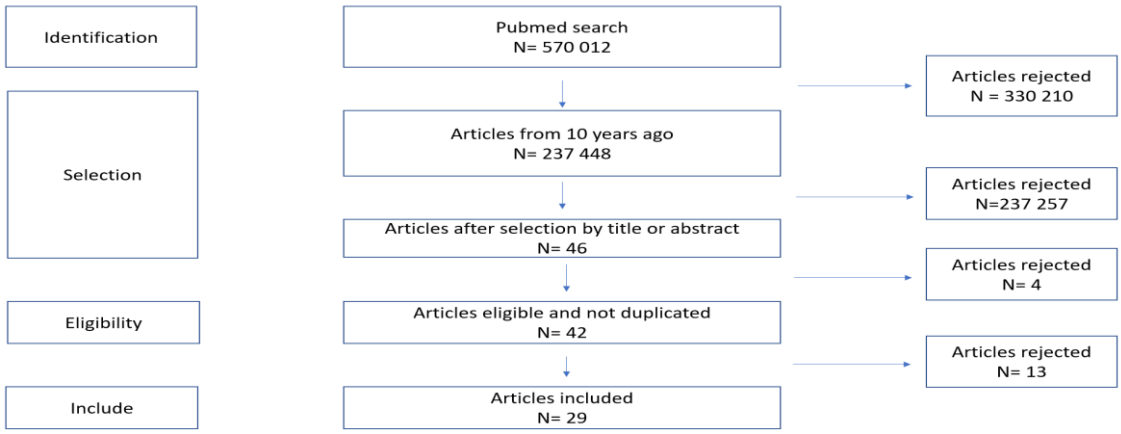
The selected articles were analyzed, and only the most relevant were used.

Although not included in the comparative table, some relevant review articles were cited to describe the state of the art.

Table 1 : PICo

Population	Everyone
Interest	Vaccine
Context	New way of prevention

Figure 1 : Flow chart of studies search and selection



4. RESULTS

570 012 articles were found throughout the search, with the use of different key words. After the elimination of articles older than 10 years ago, I kept 237 448 articles. After the selection of title and abstract, I kept 46 articles. I then rejected the duplicated ones, to get to 42 articles. From them, I only selected the most relevant ones, which made a total number of 29 articles. The software Mendeley was used to organize the references.

Relation with the date of publication:

- 3 articles published in 2021
- 1 article published in 2020
- 4 articles published in 2019
- 2 articles published in 2018
- 4 articles published in 2017
- 6 articles published in 2016
- 1 article published in 2015
- 3 articles published in 2014
- 2 articles published in 2013
- 3 articles published in 2012

Table 2: Articles

Author and date of publication	Type of article	Name of the article	Objective	Result	Conclusion
Xu *et al.* - 2021 (5)	Clinical trial	Fabrication of oral nanovesicle in-situ gel based on Epigallocatechin gallate phospholipid complex: Application in dental anti-caries	To fabricate nanovesicles in-situ gel based on Epigallocatechin gallate phospholipid complex (EGCG) in order to increase its stability and efficacy.	The formulation exhibited a strong antibacterial activity on *S. mutans*, which could reduce acid production and tooth surface adhesion. In addition, EGCG formulation could inhibit the formation of glucan and biofilm from *S. mutans* by suppressing the activity of glycosyltransferase enzymes (GTF).	EGCG-loaded nanovesicle in-situ gel holds great promise as an efficient anti-cariogenic formulation for topical oral delivery.
Al-Ansari *et al.* - 2021 (6)	Clinical trial	Synthesis of silver nanoparticles using gum Arabic: Evaluation of its inhibitory action on *Streptococcus mutans* causing	To investigate an alternative antibacterial solution from silver nanoparticles (AgNPs) against *S. mutans*.	The smaller the size, the greater the antibacterial and antiviral potential the particles exhibit. The biophysical characteristics of AgNPs are the presence of phenols, alcohols, amides, sulfoxide, flavanoids, terpenoids and steroids. The AgNPs exhibited a good antibacterial action against the oral pathogen *S. mutans*. The	The potent antibiotic action over *S. mutans* seen with the synthesized NPs, paves the way for the development of novel dental care products.

		dental caries and endocarditis		synthesized NPs at a dose level of 200g/mL exhibited an inhibition zone with 18.30 ± 0.5 nm diameter. The synthesised nanoparticles inhibited the genes responsible for biofilm formation of *S. mutans* over host tooth and gums (gtfB, gtfC, gtfD) and virulent protective factors (comDE, brpA and smu360) and survival promoter genes (gyrA and spaP, gbpB).	
Li *et al.* - 2021 (7)	Longitudinal study	Socio–Economic Disparities in Dental Health and Dental Care Utilisation Among Older Chinese	To examines disparities in dental care and in the costs of such care, according to insurance type and socio–economic status, among Chinese older adults.	28% of Chinese older adults have no remaining teeth and only 19% had used dental care in the past year. The uninsured and those with rural resident insurance had edentulousness rates of 31%, while the edentulousness rate in those with urban employee insurance was 19%. About 13% of the uninsured study respondents and 15% of those with rural resident insurance had used dental care compared with 30% of those with urban employee insurance. Those in the	Dental care disparities in China may be reduced through increasing the proportion of the population with insurance and expanding the range of dental treatments covered by all three major insurance schemes.

				highest income and education groups and those enrolled in a plan with a lower coinsurance rate had a higher likelihood of using dental care services and spending more on dental care than did those in the lowest socio–economic groups.	
Jia *et al.* - 2020 (8)	Randomized clinical trial	Enhancing the immunogenicity of a DNA vaccine against *Streptococcus mutans* by attenuating the inhibition of endogenous miR-9	To enhance the immunogenicity of a DNA vaccine against *S. mutans* by attenuating the inhibition of endogenous miR-9 (micro ARN-9).	MiR-9 inhibited the expression of antigen protein encoded by the anti-caries DNA vaccine. So, the expression of antigen protein could be suppressed by microRNAs.	Attenuating the inhibition of endogenous miR-9 enhanced the antigen expression and immunogenicity of the anti-caries DNA vaccine.
Bai *et al.* - 2019 (9)	Clinical trial	Construction of a fusion anti-caries DNA vaccine in transgenic tomato plants for PAcA gene	To construct a fusion anti-caries DNA vaccine in transgenic tomato plants for PAcA gene and cholera toxin B subunit.	The A region of pac gene of *S. mutans* and mosaic plasmid of B subunit of cholera toxin were transferred to the tomato and their integration in the tomato genome was confirmed.	Transgenic tomatoes may provide a useful system to produce human caries antigen. The use of an oral plant vaccine is a novel concept for creating modern vaccines, which provides a platform for further research and

		and cholera toxin B subunit			development of preventive measures for caries.
Yang *et al.* – 2019 (10)	Clinical trial	Anti-caries vaccine based on clinical cold-adapted influenza vaccine: A promising alternative for scientific and public-health protection against dental caries	To test an anti-caries vaccine based on clinical cold-adapted influenza vaccine.	A longer-term protection from colonization of *S. mutans* may be observed by immunization with this new anti-caries vaccine if administered in early childhood.	The cold-adapted influenza viruses, which have been used in clinics for more than 15 years, holds a great potential for developing valid vaccines against dental caries. It should be an intranasal spray which is easy, painless, and does not require professional staff, making it more acceptable for people, especially children.
Liu *et al.* – 2019 (11)	Clinical trial	FimH as a mucosal adjuvant enhances persistent antibody response and protective efficacy of the anti-caries vaccine	To investigate whether the recombinant FimH-S.T protein could modulate immune response to anti-caries vaccine *in vitro* and *in vivo*.	The mice immunized with the mixture of FimH-S.T and PAc significantly enhanced the PAc-specific antibodies in the serum along with saliva and promoted splenocyte proliferation. Pac + FimH-S.T decreased the caries lesions formation, which provided high protective efficacy against dental caries.	Recombinant FimH-S.T could enhance specific IgA responses and protection of anti-caries vaccine, possessing mucosal adjuvant ability by activating DC2.4 via TLR4 signaling pathway.

Bi *et al.* – 2019 (12)	Clinical trial	The combinations chitosan-PAM3CSK4 and chitosan-monophosphoryl lipid A: Promising immune-enhancing adjuvants for anticaries vaccine PAc	To investigate the effect of two adjuvant combinations of chitosan + Pam3CSK4 and chitosan + monophosphoryl lipid (MPL) in the immune responses to the PAc protein *in vivo* and *in vitro*.	Compared with PAc alone, Pac + chitosan + Pam3CSK4 or Pac + chitosan + MPL promoted significantly higher PAc- specific antibody titers in serum and saliva, inhibited *S. mutans* colonization onto the tooth surfaces and endowed better protection effect with significantly less caries activities. Chitosan + Pam3CSK4 and chitosan + MPL showed no statistic differences.	The combination of chitosan + Pam3CSK4 or chitosan + MPL is promising for anti-caries vaccine development.
Alam *et al.* – 2018 (13)	Clinical trial	Synthetic antigen-binding fragments (Fabs) against *S. mutans* and *S. sobrinus* inhibit caries formation	To test if synthetic antigen-binding fragments (Fabs) against *S. mutans* and *S. sobrinus* inhibit caries formation.	Fabs inhibited sucrose-induced *S. mutans* and *S. sobrinus* biofilm formation *in vitro* and a combination of *S. mutans* and *S. sobrinus* Fabs prevented dental caries formation in a rat caries mode.	*S. mutans* and *S. sobrinus* Fabs could be used in passive immunization strategies to prevent dental caries. This strategy may be applied towards a caries therapy, whereby Fabs are topically applied to the tooth surface.
St. Michael *et al.* – 2018 (14)	Clinical trial	Investigating the candidacy of the serotype specific rhamnan polysaccharide based	To examine the vaccine candidacy of the serotype specific polysaccharides elaborated by *S. mutans*.	Development of an opsonophagocytic assay which illustrated the ability of the post-immune sera to facilitate opsonophagocytic killing of the homologous and heterologous	Glycoconjugates of the rhamnan polymers of *S. mutans* are a potential vaccine candidate to target dental caries and other sequelae following

		glycoconjugates to prevent disease caused by the dental pathogen *Streptococcus mutans*		serotypes at titers consistent with the structural homologies.	the escape of *S. mutans* from the oral cavity.
Batista *et al.* – 2017 (15)	Clinical trial	LT adjuvant modulates epitope specificity and improves the efficacy of murine antibodies elicited by sublingual vaccination with the N-terminal domain of *Streptococcus mutans* P1	To evaluate the immunogenicity, protective efficacy and peptide-based immune signatures of antibodies raised in mice after sublingual immunization with a recombinant form of the P1(aka AgI/II, PAc) adhesin (P139-512) of *S. mutans.*	Sublingual administration of P139-512 in combination with the mucosal adjuvant LTK4R (a derivative of heat-labile LT toxin) induced strong and long-lasting systemic and mucosal immune responses. Incorporation of the adjuvant resulted in an enhancement of the anti-adhesive and anti-colonization activity against *S. mutans* as evaluated both under *in vitro* and *in vivo* conditions. Incorporation of the adjuvant to the vaccine formulation also changed the epitope specificity of the induced antibodies as determined by immunological signatures of sera collected from vaccinated mice. The	The sublingual administration of a P1-based subunit vaccine represents a promising approach for the prevention of dental caries caused by S. *mutans*. The role of adjuvants on the epitope specificity and functionality of antibodies raised by subunit vaccines was disclosed.

				use of a peptide microarray library led to the identification of peptide targets recognized by antibodies in serum samples with enhanced anti-adhesive effects.	
Jiang *et al.* – 2017 (16)	Clinical trial	Enhanced immune response to a dual-promoter anti-caries DNA vaccine orally delivered by attenuated *Salmonella typhimurium*	To determine if the CMV-nirB promoter immune regime was superior to nirB promoter used alone in inducing a protective immunity against *S. mutans* colonization.	The dual-promoter formula in the *Salmonella*-based DNA vaccine pCN-SS/SG was successful in inducing a mucosal immune response against *S. mutans*. Such dual promoter system (CMV-nirB) showed a promising prospect for taking advantage of mucosal immunization against dental caries as well as against other related diseases.	The effectiveness of a dual-promoter strategy in the anti-caries DNA vaccine when employing attenuated *Salmonella* as delivering vehicle for mucosal immunization was verified.
Esberg *et al.* - 2017 (17)	Clinical trial	*Streptococcus Mutans* Adhesin Biotypes that Match and Predict Individual Caries Development	To investigate if adhesin types of *S. mutans* with sucrose-independent adhesion to host Deleted in malignant brain tumors 1 (DMBT1) (*i.e.* SpaP A, B or C) and collagen (*i.e.* Cnm, Cbm) match and predict	The presence of SpaP B and Cnm subtypes coincided with increased 5-year caries increment, and their binding to DMBT1 and saliva correlated with individual caries scores. The SpaP B subtypes are enriched in amino acid substitutions that coincided with caries and binding and specify biotypes of *S. mutans* with increased acid tolerance.	This study emphasizes that careful sequence analysis and evaluation of *S. mutans* genotypes in concert with dental caries incidence serves as a useful example and model to better understand strain variation and disease association in relation to bacterial virulence in chronic

			individual differences in caries development.		infections. These results highlight the importance of developing novel approaches to diagnose high-risk patients and improve prevention and treatment of chronic infectious disease. The findings may also have relevance beyond dental caries and translate to improved systemic health.
McLaren *et al.* - 2017 (18)	Clinical trial	Exploring the short-term impact of community water fluoridation cessation on children's dental caries: a natural experiment in Alberta, Canada	To explore the short-term impact of community water fluoridation cessation on children's dental caries, by examining change in caries experience in population-based samples of schoolchildren in two Canadian cities, one that discontinued community	It was observed a worsening in primary tooth caries (deft) in Calgary and Edmonton, but changes in Edmonton were less consistent and smaller. This effect was robust to adjustment for covariates available in 2013/14 and was consistent with estimates of total fluoride intake from biomarkers from a subsample. This finding occurred despite indication that treatment activities appeared better in Calgary. The worsening was not observed for permanent teeth. For prevalence estimates only (% with >0 deft or	Trends observed in caries rates in Calgary, Canada (especially in primary teeth), along with other information gathered, appear to be broadly consistent with an adverse effect of community water fluoridation cessation. It is important to undertake subsequent oral health surveys to monitor and confirm these trends over time.

			water fluoridation and one that retained it	DMFT), the three data points in Calgary suggest a trend that, though small, appears consistent with an adverse effect of fluoridation cessation	
Sun *et al.* – 2017 (19)	Clinical trial	Flagellin-rPAc vaccine inhibits biofilm formation but not proliferation of *S. mutans*	To investigate if the antibody response induced by KF-rPAc could inhibit biofilm formation besides the adherence of *S. mutans* to salivary glycoproteins.	Both serum and saliva from KF-rPAc immunized rats significantly inhibited biofilm formation. Moreover, with the presence of serum or saliva, the biofilm formation was negatively correlated with the level of rPAc-specific antibody, and positively correlated with caries scores in rat. Additionally, in immunized mice, the level of rPAc-specific antibody was also negatively correlated with the biofilm formation. Unlike ampicillin, serum of KF-rPAc immunized mice only inhibited biofilm formation but not proliferation.	Besides the well-known blocking adherence of *S. mutans* to salivary glycoproteins by rPAc-specific antibody, flagellin-rPAc vaccine could also protect teeth from caries by inhibiting biofilm structure formation in between bacteria.
Ferreira *et al.* – 2016 (20)	Clinical trial	Sublingual immunization with the phosphate-	To observe the induction of protective immunity to *S. mutans* tooth	Mice immunized with the vaccine formulation induced specific systemic and	*S. mutans* PstS is a potential target antigen capable of inducing specific

		binding-protein (PstS) reduces oral colonization by *Streptococcus mutans*	colonization after sublingual immunization of mice with vaccine formulations containing a recombinant form of the PstS protein, with a derivative of the heat-labile toxin (LT) in combination with a mucosal adjuvant.	secreted immune responses and controlled the adhesion of *S. mutans* to the oral cavity.	and protective antibody responses after sublingual administration.
Li *et al.* – 2016 (21)	Clinical trial	Enhancement of immunogenic response and protection in model rats by CSTM nanoparticles anticaries DNA vaccine	To construct anticaries DNA vaccine and evaluate its ability to elicit mucosal and systemic immune responses in rats.	Significantly higher specific IgG antibody titers were observed in rats immunized with nanoparticles compared with rats immunized with naked pVAX1-wapA. Anti-WapA IgA and IgG antibody levels after intranasal immunization were significantly higher than those following intramuscular delivery of nanoparticles or naked pVAX1-wapA. Furthermore, fewer enamel, slight dentin and dentin moderate lesions were	WapA is an excellent candidate for anticaries vaccine development and nanoparticles as an effective delivery system.

				observed in rats immunized with nanoparticles.	
Yan *et al.* – 2016 (22)	Clinical trial	CCL17 combined with CCL19 as a nasal adjuvant enhances the immunogenicity of an anti-caries DNA vaccine in rodents	To determine whether co-administration of CCL17 and CCL19 could enhance the immunogenicity of an anti-caries DNA vaccine, pCIA-P, in rodents.	Co-administration of the CCL17 and CCL19 genes in mice caused a greater increase in the number of mature DCs in the spleen and DLNs compared with administration of CCL17 or CCL19 genes alone. CCL17 and CCL19 double-adjuvant plus pCIA-P induced significantly higher levels of anti-PAc salivary IgA and anti-PAc serum IgG antibody in mice and strengthened the ability of pCIA-P in inhibiting the colonization of *S. mutans* on rat tooth surfaces. The caries activity of the combined adjuvant group was significantly lower than that of the pCCL17/VAX or the pCCL19/VAX group.	A nasal adjuvant consisting of a combination of CCL17 and CCL19 attracts more mature DCs to secondary lymphoid tissues, inducing enhanced antibody responses against the anti-caries DNA vaccine pCIA-P and reducing *S. mutans* infection in rodents than CCL17 or CCL19 alone.
Bachtiar *et al.* – 2016 (23)	Clinical trial	Biological and Immunogenicity Property of IgY Anti *S. mutans* ComD	To elucidate the effect of IgY anti ComD on the biological properties of *S. mutans*. ComD is an	The ComD antibody was successfully induced in the hens' eggs. It inhibited biofilm formation by all *S. mutans* isolates. In addition, the expression of some protein	IgY anti-*S. mutans* ComD reduces biofilm formation by this bacterium and alters the protein profile of *S. mutans*.

			interspecies quorum-sensing signaling receptor that plays an important role in biofilm formation by *S. mutans*.	bands was affected after exposure to the antibody.	
Colombo *et al.* - 2016 (24)	Clinical trial	Relationship between the IgA antibody response against *Streptococcus mutans* GbpB and severity of dental caries in childhood	To explore the associations between the severity of dental caries in childhood, mutans streptococci (MS) levels and IgA antibody response against *S. mutans* GbpB. Moreover, other caries-related etiological factors were also investigated.	Severe-early childhood caries (S-ECC) children had reduced family income compared to those with early childhood caries (ECC) and CF. There was difference between CF and caries groups (ECC and S-ECC) in MS counts. Positive correlations between salivary IgA antibody response against GbpB and MS counts were found when the entire population was evaluated. When children with high MS counts were compared, S- ECC group showed significantly lower IgA antibody levels to GbpB compared to CF group. This finding was not observed for the ECC group.	Children with S-ECC have reduced salivary IgA immune responses to *S. mutans* GbpB, potentially compromising their ability to modify MS infection and its cariogenic potential. Furthermore, a reduced family income and high levels of MS were also associated with S-ECC.

Bao *et al.* – 2015 (25)	Clinical trial	Flagellin-PAc fusion protein inhibits progression of established caries	To investigate the therapeutic effect of a mucosal vaccine of flagellin-PAc fusion protein (KF-rPAc) against dental caries by using a new immunization protocol on the dental caries progression in rats with prior implant of *S. mutans* into their oral cavities.	KF-rPAc by nasal immunization can promote PAc-specific systemic and mucosal antibody responses and inhibit dental caries progression efficiently after the implant of *S. mutans* into the oral cavity of the rats. The rats immunized with KF-rPAc exhibited 53.9% caries reduction compared with the sham-immunized rats.	KF-rPAc could be used as an anticaries therapeutic mucosal vaccine.
Batista *et al.* – 2014 (26)	Clinical trial	Immunogenicity and *in vitro* and *in vivo* protective effects of antibodies targeting a recombinant form of the *Streptococcus mutans* P1 surface protein	To investigate the immunological features of P139-512 after parenteral administration of mice in combination with different adjuvants: alum, a derivative of the heat	Recombinant P139-512 preserves relevant conformational epitopes as well as salivary agglutinin (SAG)-binding activity. Co-administration of adjuvants enhanced anti-P1 serum antibody responses and affected both epitope specificity and immunoglobulin subclasses switching. Importantly, P139-512–specific antibodies raised in mice	These findings confirm the utility of P139-512 as a potential candidate for the development of anti-caries vaccines, and as a tool for functional studies of *S. mutans* P1.

			labile toxin (LT), and *Salmonella flagellin* (FliCi).	immunized with adjuvants showed significantly increased inhibition of *S. mutans* adhesion to SAG, with less effect on SAG-mediated bacterial aggregation, an innate defense mechanism. Oral colonization of mice by *S. mutans* was impaired in the presence of anti-P139-512 antibodies, particularly those raised in combination with adjuvants.	
Li *et al.* – 2014 (27)	Clinical trial	*Streptococcus mutans* Wall-Associated Protein A Promotes TLR4-Induced Dendritic Cell Maturation	To observe the effects of WapA on DCs (dentritic cell).	WapA could be recognized by DCs by certain specific receptors and promote the maturation of DCs through increasing TLR4-induced NF-jBand MAPK activation.	WapA is recognized by DCs and promotes DC maturation, which puts new lights on the function of WapA.
Su *et al.* – 2014 (28)	Clinical trial	Intranasal co-delivery of IL-6 gene enhances the immunogenicity of	To investigate the effects of co-delivering IL-6 expressing plasmid pCI-IL-6 on the immunogenicity of the anti-caries DNA	Marked expression of IL-6 was found in COS-7 cells transfected with pCI-IL-6. In the pCI-IL-6 co-immunized mice, the specific IgG antibodies in serum and sIgA antibodies in saliva were significantly higher than those in	Intranasal co-delivery of IL-6 gene significantly enhances the immunogenicity of the anti-caries DNA vaccine.

		anti-caries DNA vaccine	vaccine pCIA-P, which encodes the surface protein antigen PAc of *S. mutans*.	the control mice at weeks 4 and 8. Moreover, the secretion of IFN-γ from splenocytes in response to re-stimulation with PAc protein was significantly higher in the pCI-IL-6 co-immunized mice than that in the control mice, whereas the secretion of IL-4 had no significant difference. The proliferation of splenocytes from the pCI-IL-6 co-immunized mice was significantly higher than that from the mice immunized with pCIA-P and pCI vector. In the rat caries model, the pCI-IL-6 co-immunization rats displayed lower caries scores than the control rats.	
Chen *et al.* – 2013 (29)	Clinical trial	Enhanced Nasal Mucosal Delivery and Immunogenicity of Anti-Caries DNA Vaccine through Incorporation of Anionic Liposomes in	To report a new designed nanoparticle system through incorporating anionic liposomes (AL) into chitosan/DNA (CS/DNA) complexes.	The AL/CS/DNA induced a significantly (p,0.01) higher level of secretory IgA (SIgA), and a longer-term mucosal immunity than the CS/DNA in animal study. On the other hand, the AL/CS/DNA exhibited minimal cytotoxicity.	The developed nanoparticles offer a potential platform for DNA vaccine packaging and delivery for more efficient elicitation of mucosal immunity.

		Chitosan/DNA Complexes			
Yan *et al.* – 2013 (30)	Clinical trial	Co-delivery of ccl19 gene enhances anti-caries DNA vaccine pCIA-P immunogenicity in mice by increasing dendritic cell migration to secondary lymphoid tissues	To investigate how co-delivery of the gene encoding C–C chemokine ligand-19 (CCL-19) affected the systemic immune responses to an anti-caries DNA vaccine pCIA-P in mice.	The expression level of CCL19-GFP fusion protein was considerably increased 48 h after transfection of COS-7 cells with pCCL19/GFP plasmids. The fusion protein showed potent chemotactic activity on DCs *in vitro*. The level of serum PAc-specific IgG was significantly increased from 4 to 14 weeks in the mice vaccinated with pCIA-P plus pCCL19/GFP. Compared to mice vaccinated with pCIA-P alone, the splenocytes from mice vaccinated with pCIA-P plus pCCL19/GFP produced significantly higher level of IFN-γ, but IL-4 production had no significant change. Following intramuscular co-delivery, pCCL19/GFP plasmid and fusion protein were detected in the spleen and draining lymph nodes. Administration of CCL19 gene in mice	CCL19 serves as an effective adjuvant for anti-caries DNA vaccine by inducing chemotactic migration of DCs to secondary lymphoid tissues.

				markedly increased the number of mature DCs in secondary lymphoid tissues.	
Shi *et al.* – 2012 (31)	Clinical trial	Flagellin enhances saliva IgA response and protection of anti-caries DNA vaccine	To analyze the effects of FliC protein on the serum PAc-specific IgG and saliva PAc-specific IgA antibody responses, the colonization of *S. mutans* on rat teeth, and the formation of caries lesions.	FliC promoted the production of PAc-specific IgG in serum and secretory IgA (S-IgA) in saliva of rats by intranasal immunization with pGJA-P/VAX plus FliC. Enhanced PAc-specific IgA responses in saliva were associated with the inhibition of *S. mutans* colonization of tooth surfaces and endowed better protection with significant fewer caries lesions.	FliC could enhance specific IgA responses in saliva and protective ability of pGJA-P/VAX, providing an effective mucosal adjuvant candidate for intranasal immunization of an anti-caries DNA vaccine.
Sun Y et al. – 2012 (32)	Clinical trial	Flagellin-PAc fusion protein is a high-efficacy anti-caries mucosal vaccine	To evaluate the ability of KF-rPAc to promote an rPAc-specific salivary IgA antibody response and protective efficacy against caries in rat.	KF-rPAc promoted significantly higher rPAc-specific antibodies in serum as well as in saliva than did an equivalent dose of rPAc alone or a mixture of KF + rPAc. Intranasal immunization of 8.5 μg KF-rPAc could achieve 64.2% reduction of dental caries in rats.	Flagellin and PAc fusion strategy is promising for anti-caries vaccine development, and KF-rPAc could be used as an anti-caries mucosal vaccine.
Kim *et al.* – 2012	Clinical trial	A monoclonal antibody specific to	To investigate the inhibitory activity of the	A hybridoma cell line, HBN8, that produced MAb against the GTFBN protein of *S. mutans*	The anti-GTFBN antibody could be used as a vaccine to prevent the

(33)		glucosyltransferase B of Streptococcus *mutans* GS-5 and its glucosyltransferase inhibitory efficiency	monoclonal antibody against the formation of insoluble glucans that are important in *S. mutans* and other oral bacteria for bacterial tooth surface attachment and the formation of dental plaque.	GS-5 was established. This MAb could inhibit the glucan-producing activity of crude GTFs in a dose-dependent manner.	aggregation of *S. mutans* on teeth surfaces, and thus prevent the formation of dental caries.

5. DISCUSSION

a. The caries

Dental caries is a multifactorial disease and a dynamical fluctuating disease process (1). Modern diet has changed the morphology of our dentition, which was different some millennia ago. New high sugary diet is softer and more liquid than before, so our teeth are less required, which might explain why teeth can be rapidly lost (1), and why caries are more likely to be found in developed countries rather than in developing countries. Even though sex and ethnic differences can be a factor, it is still minor compared to sugar consumption, lifestyle and economic differences (1).

Frequent ingestion of carbohydrate (sugar), poor oral hygiene and inadequate exposure tends to favor more caries (1).

i. Mechanisms

The mechanisms and pathophysiology underlying the development of dental caries are now extremely well understood. It occurs by a dynamic process that involves repeated cycles of demineralization and remineralization (1).

1. Demineralization

Bacteria from the biofilm produces acid from the metabolization of fermentable carbohydrates, which demineralizes crystalline mineral structure. Caries usually initiates below the enamel surface (1).

2. Remineralization

The saliva then neutralizes the acids, making the biofilm's pH neutral and saturated with fluoride, phosphate and calcium. (1)

3. Lesion

If demineralization does not stop, the mineral loss is greater in the subsurface than in the surface, creating a lesion at subsurface level. A white spot can appear if there is sufficient mineral

lost. But if the causative factors are modified or preventive measures are used, the lesion can be stopped or reversed. For example, we can use fluoride on caries in early stages (subclinical).

Maintaining the mineral homeostasis is the key to keep a healthy tooth. (1)

4. International Caries Detection and Assesement System (ICDAS)

It's an evidence-based system for detection and classification of caries.

The codes 1 and 2 are already considered demineralized. They can stop and remain as an inactive lesion with application of fluoride, changes in the diet and in the local ecology.

The codes 3 and 4 are used if the surface porosity increases with micro cavitation on the enamel or in the surface dentine layer for root caries, then

The codes 5 and 6 are used when there is a physical cavitation, and the dentin can be seen. The lesion can still be stopped but, if it continues, the pulp can be compromised. In this case the only options are extraction or root canal. (1)

ii. Microbiome

The wild living animal species have a harmonious balance between their oral microbiome and them, and we should also find a way to have such a harmonious co-existence despite our modern-day lifestyle. To eliminate the oral microbiome is not the solution.

Human oral microbiome has greatly changed since the Neolithic period (12,000 to 7,000 years ago). First, humans had a hunter-gatherer diet, then a farming lifestyle to a sugary industrialized diet around 1850s, to finish with modern processed foods, smoking habits, antimicrobials and vaccines. Those changes put our microbiome into a disease-associated configuration, making the oral microbiome less diversified, "*less resistant to perturbation and with a greater susceptibility to the insertion of pathobionts or even true pathogens in the microbial community*". (2)

1. Caries microbiome

"On caries active and caries-free individuals, the supragingival microbiome is approximately the same, at around 50%. Only 10 genera were expressed in high abundance, including Streptococcus spp., Veillonella spp and Actinomyces spp. S. mitis (25.5%) and S. sanguinis (9.1%) were predominant. S. mutans (1.2%) was a comparatively minor constituent"

(2). These studies suggest that the caries is more complex and multi-faceted than what is thought.

Glycoprotein from the salivary film facilitates biofilm formation, by interacting with adhesins.

"S. mutans is only associated with caries initiation (white spots) but not with caries progression. S. mutans appears to have the characteristics of a keystone pathogen or of a pathobiont driven by a changing dietary environment" (2).

2. Streptococcus mutans

S. mutans was discovered in 1924 by J. Clarke. It lives in the dental plaque, thanks to the three GTF it produces (namely GtfB, -C and -D), synthesizing glucose polymers of glucan. It also encodes some GBP (A, B, C and D). Its adhesins enable colonization even without sucrose. The dual antigen I/II, also named P1, SpaP or Pac, is one of the most studied antigens. Indeed, P1 deficient mutant demonstrates reduced binding to saliva, aberrant biofilm formation and a reduced cariogenicity in a rat caries model. GTF, GBP and adhesive glucans promote accumulation of microbial cells and form a polymeric matrix which protects the embedded bacteria. (34)

S. mutans is one of the main targets for caries prevention. Some products, such as propolis, curcumin, cranberry, and green tea extracts seem to be effective against *S. mutans* but aren't selective towards it. (34) There are other promising approaches against *S. mutans* as described by Al-Ansari *et al.* who showed that AgNPs antibiotic can also affect *S. mutans* (6). Xu *et al.* also showed that EgCG nanovesicles suppress the activity of GTF, inhibiting the formation of glucan and biofilm from *S. mutans*. (5)

3. Immunity against caries

"Individuals with low or non-detectable levels of Mutans streptococci early in life remain caries-free into adulthood" (2). Early oral colonization and colonized individuals experience are inversely related to salivary IgA antibody. Bacterial adhesin, GTF and GBP are the main targets.

"Early in childhood, children begin to synthesize serum IgG antibody to Mutans streptococcal antigens, followed in time by production of IgA. Serum IgG antibody levels increase during childhood and remain detectable throughout life. In young adults, anti-S. mutans IgG titers are inversely related to disease levels. In older adults, serum IgG antibody to cariogenic streptococci is directly related to cumulative dental caries experience, whereas IgA levels are

inversely related. This reciprocal relationship between IgA and IgG responses throughout life indicates that the initial adaptive immune responses to mutans streptococcal antigens may influence the time and rate at which these streptococci join the biofilms of the primary dentition" (2).

Colombo *et al.* showed that children having S-ECC have reduced salivary IgA immune response to *S. mutans.* (24)

iii. Prevention

"The goals of dental caries prevention are to preserve sound tooth structure, to prevent demineralization of enamel and to promote natural healing processes" (1). There are several different ways to prevent caries formation. Some are basic, like education of the people, by promoting oral hygiene and encouraging less sugar consumption. Other methods include the fluoridation of community water, salt and milk. A study by McLaren *et al.* done in Calgary, Canada, in 2017 showed that fluoridation of community water has negative effects over the population if stopped, proving that prevention can truly have a positive effect over the population oral health. (18)

b. The vaccine

Vaccination is one of the most important discoveries of medicine. Its purpose is "*to induce a protective immune response to the targeted pathogen without the risk of acquiring the disease and its potential complications"* (4). By doing that, it will mimic the natural interaction of the pathogen with the immune system, creating an immune memory.

The first line of defense of an organism is the innate immunity. It is not specific and it has no memories, taking only a few hours to be established.

The second line of defense is the adaptive immunity. Lymphocytes and antibodies can recognize and eliminate all know pathogens. This appears at a later stage of the infection.

The antigen-presenting cells or APC comprise dendritic cells (DC), macrophages, B lymphocytes or B cells. Through their major histocompatibility complex (MHC) class II, they present the antigen, which will be recognize by the lymphocyte T CD4 (LTCD4).

A vaccine needs to have three major characteristics:

- Being efficient (inducing long duration protection and immune memory)
- Being safe

- Being easy to administrate (modality and number of administration doses) (3,4)

i. The different types of vaccines

1. Live attenuated vaccine

The vaccine uses the pathogen, which is weakened, altered or selected to be less virulent, by passing the pathogen through a series of in vitro cell cultures, allowing the pathogen to only mimic the wild pathogen in a very soft way, causing an attenuated or asymptomatic disease to stimulate the immune response.

Its main propriety is to confer an immunogenicity almost as if the person got the wild pathogen. The immunogenicity is achieved between 10 and 14 days. It does not need adjuvant and it only needs a small number of doses (2 or 3). It is injected in a sub-cutaneous way.

This type of vaccine is contraindicated in pregnant women because of fetal infection causing congenital disease, and in immunocompromised individuals, causing death in case of unregulated pathogen replication (however, it can be used in some cases after a critical benefit-risk assessment). It should be noted that, in very rare occasions, the virus can revert to a form able to cause the disease. (3,4)

2. Non live vaccines

The vaccines don't contain any living or infectious particles and are very safe (even for immunocompromised people or pregnant women).

a. Inactivated vaccine

The vaccine contains the totality of the bacteria or viral particles inactivated by chemicals, radiation or thermal methods. The inactivation consists of destroying the ability of the pathogen to replicate while keeping the immunogenicity, allowing the system to recognize it.

It needs multiple administration to have a long-term protection, and some adjuvants. (3,4)

b. Subunit vaccine

In this case, the vaccine contains a selected active fragment of the pathogen inducing vaccinal immunity. The fragment may be a polysaccharide, a protein, or the part of it that may form virus-like particles (VLP).

It needs adjuvant, the immunogenicity is lower, and the person needs multiple primo injection plus booster doses during his life.

There are different types of subunit vaccine, such as protein vaccine, toxoid, VLP, polysaccharide and polysaccharide conjugate vaccine.(3,4)

ii. Adjuvants

They are needed to induce a strong and long immune response. They enhance and amplify the initial immune response, especially in population having low response levels such as immunocompromised, elderly and infant. They also reduce the number of doses. (3,4)

iii. Future vaccines

1. DNA/RNA vaccine

One of the most promising ways of making vaccines is to insert "*DNA or RNA that encodes antigenic proteins into body cells (e.g. muscle or skin cells), which induces antigen presentation to the immune system, triggering an immune response*" (4).

There are many licensed DNA vaccines for animals, but only one for humans, the ZyCoV-D, made by the Indian Cadila Healthcare laboratory and allowed on the 20th of august 2021 by the Drugs Controller General of India, DCGI. Its efficacy is 66,6% against clinical form of Covid and 100% against serious forms. (35)

2. Vectored vaccine

It combines live and subunit vaccines advantages. It is made from *"non-pathogenic infectious viruses expressing antigenic protein genes of a pathogen. The viral vectors are derived from retroviruses, herpes simplex viruses, adenoviruses or poxviruses, and have been developed for vaccination against a wide array of pathogens"*.(4)

The main problems are an early vaccine clearance and reduced immunogenicity. This is due to high prevalence from pre-exposure to the virus, leading to neutralizing antibodies against the vectors. (4)

3. New type of injection

The conventional routes of administration are intramuscular, subcutaneous and intradermal injections. But new methods are being developed to improve vaccine acceptance among people who are afraid of needles, making it more comfortable. These methods may be through skin, using microneedle or needle-free devices, or through mucosal tissues (oral, sublingual or intranasal).

Systemic and mucosal immune responses should also be induced at the site of pathogen entry (4).

c. The anti-caries vaccine

i. Types of vaccines

As DNA vaccines are very promising, the research about making an anti-caries DNA vaccine is documented in this work. In 2019, Bai *et al.* found that transgenic tomato can be used to produce antigens for an anti-caries DNA vaccine. (9)

The subunit vaccine is also studied. In fact, the sublingual administration of a P1-based subunit vaccine represents a promising approach for the prevention of dental caries caused by *S. mutans* (26).

Mucosal vaccine could use Flagellin and PAc fusion strategy as a promising way (32), or KF-rPAc (25).

The passive immunization strategy consists of administering *ready-made antibodies, which circulate in the body and impart specific protection* (36). It has been discovered that *S. mutans* and *S. sobrinus* Fabs could be used for the development of the vaccine (13).

ii. The different adjuvants

We can observe that articles from around 10 years ago tried to focus more on the research of an adjuvant, as result FliC protein, or CCL19 are potential effective candidate (30,31). In 2016, Yan *et al.* tried the co-administration of CCL19 and CCL17 as a nasal adjuvant, claiming that it could also be a good candidate (22). The role of adjuvants on the epitope specificity was also disclosed by Batista *et al.* in 2017 (26). More recently, the combination of chitosan + Pam3CSK4 or chitosan + MPL was considered promising (11).

iii. The delivery systems

There are many delivery vehicles depending on the type of vaccine. Attenuated *Salmonella* can be used for mucosal immunization (16).

Intranasal spray is one of the best ways. It's easy to administer, painless, and does not require professional staff, which makes it more acceptable for people, especially children (10).

In 2013, Chen *et al.* discovered that nanoparticles can be useful for packaging and delivering DNA vaccines. And, in 2016, Li *et al.* confirmed that nanoparticles are an effective delivering system (21,29).

iv. The inhibition of biofilm formation or aggregation of *S. mutans*

Some research works are about preventing the aggregation of *S. mutans* on tooth surfaces. This could be done by the anti-GTFBN antibody.

Other studies are about reducing or inhibiting the biofilm, by using IgY anti-*S. mutans* ComD, altering the protein profile of *S. mutans* (23), or by using flagellin-rPAc vaccine, blocking the adherence of *S. mutans* to salivary glycoproteins (19).

In 2016, Ferreira *et al.* stated that *S. mutans* PstS is a potential target antigen, inducing specific protective antibody after sublingual administration (20).

v. The enhancement of immunogenicity

Some articles showed that the immunogenicity can be enhanced, for example with the intranasal co-delivery of IL-6 expressing plasmid pCI-IL-6 or with the combination of CCL17 and CCL19 as a nasal adjuvant (22,28). More recently, Liu *et al.* showed that recombinant FimH-S.T could enhance specific IgA responses and protection of anti-caries vaccines (11). Additionally, Jia *et al.* demonstrated that attenuating the inhibition of endogenous miR-9 enhanced the antigen expression and immunogenicity of the anti-caries DNA vaccine (8).

In 2014, Batista *et al.* showed that WapA is recognize and promotes DC maturation, and, in 2016, Li *et al.* confirmed that WapA is an excellent candidate for the vaccine (21,27).

vi. Other potential candidates

Over the years, many promising potential candidates for the development of the vaccine were found. One of them is the glycoconjugates of the rhamnan polymers of *S. mutans* to target

dental caries (14). Another one is the cold-adapted influenza virus, which could be used to develop the vaccine (10).

d. Global point of view

i. For which population?

"In 2010, untreated caries in deciduous teeth was the tenth most prevalent health condition, affecting 9% of the global child population" (37).

"Untreated caries in permanent teeth was the most prevalent health condition in 2010, affecting 35% of the global population, or 2,4 billion people worldwide" (37).

Caries experience or untreated caries lesions are significantly associated with lower socioeconomic position. Indeed, extreme oral health inequalities existed for this classes and marginalized groups (homeless, prisoners, refugees, indigenous groups and people with long-term disabilities…). In the USA, "*prisoners had 8,4 times more untreated caries than non-institutionalized US adults"* (37). Disabled persons have more untreated caries than the rest of the population.

A study conducted by Li *et al.* in 2021 showed that "*almost 30% of Chinese seniors have no remaining teeth, and that only 19% of elderly Chinese had visited a dentist in the past year"* (7). Old people are also a population more subject to caries than other groups. Plus, they tend to go less often to the dentist, waiting for a potential caries to become more important, which makes them a targeted population for the vaccine.

School absenteeism due to caries treatments can greatly affect school performance, exacerbating social inequalities. It also affects the quality of people's life (adults and children) because of the pain, problems with chewing, biting and eating. Additionally, it affects social and family activities, making the emotions expression more difficult (37).

ii. Economy

Caries inflicts high costs on the society, not only by the direct (the treatments), but also by the indirect (productivity losses due to absence from school or work) costs. "*In the 28 EU member states in 2015, dental diseases (€90 billion) ranked third behind diabetes (€119 billion) and cardiovascular diseases (€111 billion)"* (37).

Caries prevention has a high cost for both the individual and the public health system.

Even if preventive strategies are cost effective and money saving to the public health system, they´ll still have a great impact on the economy for the time it takes to be effective (1).

Despite the major cost, oral diseases are rarely seen as a health priority, making the dentistry profession marginalized in health policy (37).

In China, among the people with rural insurance, 31% are edentulous and, among the urban insured, 19% are edentulous. The urban area shows more people with teeth remaining than the rural area, perhaps because the salaries are higher in urban area, allowing people access to health care more easily. Additionally, there might be a higher rate of dentist studios in these areas (7).

iii. Limitations

Nowadays, most of the studies are focused on short-term comparison of preventive ways such as comparing fissure sealant and conventional filling. However, studies on the long-term costs are still lacking. "*Few robust studies looking at the costs and benefits of using behavioral change techniques to modify caries risk or of using anticipatory guidance or modifying oral health literacy exist. These topics need further research across disciplines*" (1).

Moreover, the anti-caries vaccine doesn't exist yet. Studies comparing the potential cost saving, along with more traditional prevention methods, are needed. So, we can ask ourselves if the public services will save money for a short-, mid- or long-term period.

Knowing that oral health care isn't a priority, we can ask ourselves if the governments around the world would agree to support the vaccination costs for their populations. We can also wonder if they will make the vaccine available for everyone or only a part of the population.

One of the major challenges is to fight back the adversity for vaccination, knowing that people can be very mistrustful regarding vaccines, as we have seen during the Covid-19 pandemic. Will the vaccine be accepted by the population?

Will the vaccine change the dentistry? Access to an effective vaccine will dramatically reduce the incidence of caries. Is it possible that the reduction of dentists' workload could bring the job to its end? Are the dentists willing to accept the vaccine, knowing it could potentially bring less work for them or even make them lose their job? Will the dentists protest, to make sure the vaccine won't be used and they can stick to their job?

When will the vaccine be available, will it be in a few years or later? Will the vaccine have adverse effects?

6. CONCLUSION

The caries can affect anyone at any age, it can cause much pain and discomfort, decreasing social interaction and productivity at work. The more a person waits to treat it, the higher the cost will be, for the person and for the country. The caries is also a factor of social inequality, with lower social classes more impacted than higher ones. There is already some way of prevention, like water or salt fluoridation. An anti-caries vaccine could potentially be a good way of preventing the disease, but it also comes with some limitations, such as the time needed to develop it, the adversity for vaccination among the population, the long-term cost (which is to be determined), and the possibility of affecting the dentistry profession by decreasing its workload.

DNA, mucosal and subunit vaccines are some of the most promising types of vaccines. New adjuvants, such as the combination of chitosan + Pam3CSK4 or chitosan + MPL, FliC protein, or a co-administration of CCL19 with CCL17, are being tested. All of them showed good perspectives for the future. Delivery systems like nanoparticles or intranasal sprays have shown good results. *S. mutans* is the major target of the vaccine, which can act by preventing its aggregation, or the biofilm formation, by inhibiting or reducing it. Some trials succeeded in enhancing the immunogenicity of the vaccine. Overall, the results achieved so far have brought a good hope for the future.

However, more studies should be made to answer all the questions that are lacking a response.

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ANNEX

Table 3: Additional informations

Author and date of publication	**Type of article**	**Name of the article**
Patel – 2020 (36)	Review	Dental caries vaccine: are we there yet?
Lemos *et al.* – 2019 (34)	Review	The Biology of *Streptococcus mutans*
Peres – 2019 (37)	Review	Oral diseases: a global public health challenge
Canouï *et al.* – 2019 (3)	Review	History and principles of vaccination
Righolt *et al* – 2018 (38)	Review	Global-, Regional-, and Country-Level Economic Impacts of Dental Diseases in 2015
Vetter *et al.* – 2018 (4)	Review	Understanding modern-day vaccines: what you need to know
Kilian – 2018 (2)	Review	The oral microbiome – friend or foe?

Pitts *et al.* – 2017 (1)	Review	Dental caries
Porter *et al.* – 2017 (35)	Review	DNA Vaccine Delivery and Improved Immunogenicity
Costalonga *et al.* – 2014 (39)	Review	The oral microbiome and the immunobiology of periodontal disease and caries
Rosier *et al.* – 2014 (40)	Review	Historical and contemporary hypotheses on the development of oral diseases: Are we there yet?
Yan – 2013 (41)	Review	Salivary IgA enhancement strategy for development of a nasal-spray anti-caries mucosal vaccine
Shanmugam *et al.* – 2013 (42)	Review	Dental caries vaccine - A possible option?