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Novel technologies for the prevention and treatment of dental caries: a patent survey

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Abstract

Importance of the field: Dental caries is one of the most common preventable childhood diseases; people are susceptible to this ailment throughout their lifetime. In the United States, 90% of late adolescents and young adults have dental caries, while 94% of all dentate adults had evidence of treated or untreated coronal caries. Dental caries is often not self-limiting and without proper care, caries can progress until the tooth is destroyed.

Areas covered in this review: In this paper, the etiology of dental caries was briefly introduced. It was followed by a thorough review of patents and literatures on the recent development of various novel technologies for the prevention and treatment of dental caries.

What the reader will gain: Recent advances in anti-plaque agents, including chemoprophylactic agents, antimicrobial peptides, vaccines, probiotics/replacement therapy and sugar substitutes, and remineralization agents including fluorides and casein phosphopeptides are analyzed.

Take home massage: Both the discovery of new anti-caries agents and the development of dentotropic delivery systems will be the future focus of this research field.

1. Introduction

Dental caries is one of the most common preventable childhood diseases; people are susceptible to this ailment throughout their lifetime (1). In the United States, 90% of late adolescents and young adults have dental caries, while 94% of all dentate adults had evidence of treated or untreated coronal caries (2-4). Dental caries is often not self-limiting and without proper care, caries can progress until the tooth is destroyed (5).

The definition of dental caries is the localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates (6). Endogenous bacteria {mainly mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) and *Lactobacillus spp.*} in the biofilm (dental plaque) produce weak organic acids as metabolic by-products of fermentable carbohydrates. The acids would cause local pH to fall below a critical value resulting in demineralisation of the tooth tissue (7,8). In the demineralization process, the bacteria produced organic acids that diffuse into the tooth through the water amongst the hydroxyapatite crystals, which are the major composition of tooth enamel and dentin. When the acid reaches a susceptible site on a crystal surface, where impurities and inclusions of other ions (especially carbonate ion) incorporated in the crystal lattice producing defects and calcium deficient regions, calcium and phosphate are dissolved

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and transferred into the surrounding aqueous phase between the crystals (9). If the diffusion of calcium, phosphate, and carbonate out of the tooth is allowed to continue without proper remineralization, cavitation will eventually take place (8,10). Remineralization is the body's natural repair process for subsurface non-cavitated carious lesions (11). It is a process where calcium and phosphate either from saliva or other topical sources diffuses into the tooth and with the help of fluoride, builds on existing crystal remnants rather than the formation of new crystals (2). The rebuilt crystalline surface, however, is composed of a veneer of well-formed mineral most likely similar to fluorapatite due to the presence of fluoride, and is much more resistant to acid attack than the original structure (1,9). Demineralization and remineralization happen simultaneously in the oral cavity for most of the people. Whether dental caries development is progressive, static or reversal is dependent on a balance between demineralization and remineralization. Therefore, any factor that can push this balance toward the proceeding of remineralization can be utilized as a weapon in the battle against dental caries. In this review of relevant patents, we will discuss several novel therapeutic developments for the prevention of dental caries.

2. Therapeutics to prevent the demineralization caused by dental biofilm

Dental caries is essentially a disease caused by accumulation of acid-producing endogenous bacteria (primarily a species called Mutans Streptococci, MS) on non-shedding tooth surface. Bacteria live on tooth in microcolonies assembled in the form of dental biofilm (dental plaque). Mature dental plaque is a complex multispecies biofilm that grows on the tooth surface, embedded in a protective matrix of host and bacterial polymers including polysaccharides, proteins, and DNA secreted by the cells (5,12), which provides protection from desiccation, host defenses and predators, and provides enhanced resistance to antimicrobial agents (13). Since acid accumulation in dental plaque is the driving force for demineralization and caries development, antimicrobial approaches, including the use of antimicrobial agents, represent a valuable measure for caries control.

2.1. Chemoprophylactic agents

Chemoprophylactic agents that are used in dental caries prevention include classical antibiotics such as penicillin and vancomycin (14,15); cationic agents such as chlorhexidine and cetylpyridinium chloride (16,17); plant derived compounds such as sanguinaria extract (18); anionic agents such as sodium dodecyl sulphate (SDS) (19); and non-ionic agents such as triclosan (20). These agents are generally delivered as mouthwashes or toothpastes but can also be applied in the form of gels or varnishes to slowly release the drug and achieve prolonged inhibitory effect (21).

Chlorhexidine is one of the most tested compounds and its anti-plaque properties are well-known. In a supragingival biofilm model, chlorhexidine was shown to inhibit bacterial growth and biofilm formation (22,23). Because chlorhexidine is positively charged, it binds to various surfaces including enamel pellicle, hydroxyapatite and mucous membranes. A major part of the effectiveness of chlorhexidine is due to this substantivity (21). However, the retention of chlorhexidine on tooth surface also leads to its an undesirable side-effect which is tooth staining and calculus formation (24,25). To address this problem, an oral hygiene composition comprises chlorhexidine gluconate with an anionic anticalculus agent has been disclaimed by Barton, et al. (26). This composition is stabilized by both a non-ionic surfactant having a high hydrophilic/lipophilic balance and an amphoteric surfactant.

Triclosan is the most commonly used and most potent example of the chlorinated diphenyl ether class of antibacterial compounds (27). Several large clinical trials have shown that toothpastes containing triclosan and zinc citrate significantly reduced plaque and gingival scores (28,29). However, poor water solubility (11 μg/ml, which is lower than its MIC of 20

μg/ml) (30) and low retention (half-life for clearance is only 20 min) (31) in the oral cavity have limited its effectiveness and application for prevention and treatment of dental caries. To increase the solubility of triclosan, several approaches including triclosan emulsion product containing surfactant and emulsifier (32), triclosan rinse and dentifrice compositions containing cyclodextrin (33), and triclosan oral composition containing sodium lauryl sulfate as surfactant (34), have been developed.

One of the most common problems in dental caries chemotherapy is the inability to maintain the minimum inhibitory concentration (MIC) of the drug in oral cavity. Delivering vesicles such as dentifrices or mouth rinses introducing antimicrobial agents into the oral cavity with an initial concentration. Almost immediately, the initial concentration begins to decrease due to the dynamics of the oral cavity, and eventually drops below the MIC. Thus, there is a continues need of alternative methods for delivering antimicrobial agents to the oral cavity. Iyer, et al. (35) tried to increase the frequency of the application of antimicrobial agents to the oral cavity at an extend time by using the vehicle of chewing-gums. The chewing-gum includes two types of antimicrobial agents that release from the gum base at different rates, which maintain the concentration of at least one of the antimicrobial agents above the MIC for an extended period of time.

Longer retention time may be achieved by using polymeric delivery systems. PVA/MA is the non-proprietary designation for a polyvinylmethyl ether/maleic acid copolymer (Gantrez®). It was found to increase triclosan retention in oral cavity since 1989 (36) and its application in oral product was patented in 1990 (37). The copolymer solubilize triclosan through its methoxyether groups while binds to tooth surface through its carboxy groups (38). Both *in vitro* and *in vivo* (39,40) studies showed that using denitrifies containing both triclosan and copolymer enhanced the retention of triclosan and improved its anti-plaque efficacy when compared to triclosan alone. Plochocka (41) disclosed a similar polymeric delivery system where drug is covalently bond to the reactive group on the polymer chain, the bond is hydrolyzable in aqueous solution, therefore slowly release active drug.

Recently, a mineral-binding micellar drug delivery system was developed, which could quickly bind to the tooth surface and releases encapsulated drug over a prolonged period of time. This was accomplished by covalently conjugating the tooth-binding moieties to the ends of Pluronic copolymer using “click chemistry” (42). This approach would not only increase the water solubility of non-ionic antimicrobial drug such as triclosan, but also greatly enhance the retention of the drug on tooth surface.

More and more attentions have been attracted to natural antibacterial substances as useful antimicrobials (43). These plant-derived substances, mainly polyphenols, including extracts of miswak (44), tea tree oil (45), green tea (46,47) and manuka honey (43) have already been incorporated into products such as mouth rinses (48) to enhance their antimicrobial properties. Several possible mechanisms against *S. mutans* have been revealed during the investigation of the effect of plant extracts in the prevention of dental caries (49). Some plant extracts have been found to be able to inhibit glucosyltransferases (GTFs) activity and insoluble glucan synthesis in *S. mutans in vitro* culture (50-52) while some study showed inhibition of acid production from sucrose or glucose (53,54). There are also study showed inhibition of S. *mutans* adhesion on hard surface (55,56). However, very limited number of studies showed that the plant extracts have bactericidal activity against *S. mutans* (57). These results suggest that majority of the plant extracts showed activity against several metabolic activities of *S. mutans* and are able to reduce the virulence of this cariogenic bacterium but not the viability.

Recent advances in caries prevention using plant extracts are more focused in finding novel active extracts. Mezine, et al. (58) disclosed a composition derived from water soluble components of Labiatae family plant material. This composition is able to prevent dental plaque accumulation through inhibition of GTF enzyme activity, reduce caries-associated inflammation in the oral cavity by cyclooxygenase inhibition, and provide a strong anti-oxidative capacity. A non-food anti-microbial-adhesion and aggregation composition comprising a suitable carrier and an effective amount of an adhesion inhibitory fraction isolated from berry juice of the Vaccinium plant genus has been disclosed by Ofek, et al. (59). This adhesion inhibitory fraction is characterized as being polymeric and having a molecular weight of 14,000; an elemental analysis of carbon 43-51%, hydrogen 4-5%, no nitrogen, no sulfur and no chlorine. This composition is able to inhibit bacteria-bacteria interaction and interactions between bacteria and pellicle layer on tooth surface. A possible mechanism of this inhibitory effect might be the interruption of lectin-carbohydrate interaction whereby the sugar residues on one bacterial pair interact with a lectin on the surface of the other bacterial pair. Majeed, et al. (60) disclosed an essential oil composition derived from Coleus forskohlii showed significant inhibitory action against *S. mutans* which represents a novel natural essential oil for prevention and treatment of dental caries.

2.2. Antimicrobial peptides

One critical issue associated with the use of chemotherapy today is the progressive increase and proliferation of antibiotic-resistant organisms which significantly reduces the efficacy of conventional antibiotics (61,62). Recently, antimicrobial peptides (AMPs) have come to the forefront as potential antibiotic surrogates due to their robust killing activity against a wide spectrum of bacterial species, including drug-resistant strains. AMPs are genetically common molecules of innate immunity that have been discovered in single-cell and multicellular forms of life. These peptides may vary dramatically in peptide sequence and posttranslational modification (linear, circular, etc.), but the majority of them exhibit similar physical hallmarks including amphipathic mixtures of α-helical and ß-sheet structures and an overall cationic charge (63). Their mode of action often involves binding to the negatively charged moieties, e.g. lipopolysaccharide (LPS), on the microbial membrane. Once bound to the microbial surface, the peptides are predicted to lead to membrane disruption by insertion, but may also translocate into the microbe and kill by intracellular mechanisms (64). Due to their attraction to negatively charged structural molecules on the bacterial membrane, development of resistance to these peptides is rare (65).

It seems AMPs represent an ideal potential therapeutic agent against microbial infection, including oral cavity. However, their application has been blocked by several issues including difficulty and expense of manufacturing, and short half-lives due to proteolytic degradation (66). One thought to address this problem is to use exogenous modifiers to regulated AMP expression at the transcriptional level rather than directly apply the peptide. An active agent 1, 25-dihydroxyvitamin D3 (biologically active form of vitamin D) has been recently discovered to be able to induce the expression of the gene encoding LL-37 while cause less toxicity (host inflammatory response) (67). Another solution for the problem is to use peptide mimetic. They are a series of inexpensive nonpeptidic oligomers and polymers, modeled after compounds found in nature, that adopt amphiphilic secondary structures and exhibit potent and selective antimicrobial activity (68). A recent successful example is the development of a peptide mimetic based on the structure of magainin. In their study of this compound, meta-phenylene ethynylene (mPE), it was found to be able to prevent *S. mutans* biofilm formation at nanomolar concentrations (69). Since AMPs are widely distributed in nature, looking for effective AMPs from other sources such as plants and animals might be a good idea and can greatly reduce the cost. Reynolds, et al. (70) recently disclosed an invention of AMPs that can be derived from the milk protein casein. These peptides can be

used in foods as antimicrobial preservatives, in oral care products (e.g. toothpaste, mouthwash or dental floss) for the control of dental plaque and suppression of pathogens associated with dental caries. Leung, et al. (71) disclosed a novel AMP that composed of only 10 amino acids. Compare to the traditional large and complex AMPs, this peptide is more stable, easier to synthesis at a lower cost. Bobek (72) disclosed new AMPs with significantly increased stability by using D-isomers of MUC7-12-mer peptide of human saliva MUC7. The isomers have antimicrobial activity comparable to that of the L-isomers and are resistant to proteolysis.

In addition to the aforementioned progress, another important direction for AMP development is target-specific antimicrobial therapy. Due to the broad spectra of activity, normal flora can also be disrupted by AMPs, which could leads to secondary infections or other negative clinical consequences. By utilizing a pheromone produced by *S. mutans*, namely, the competence stimulating peptide (CSP), as a targeting domain to mediate *S. mutans*-specific delivery of an AMP domain, the AMP was potent against *S. mutans* grown in liquid or biofilm states but did not affect other oral streptococci tested (73). Li, et al. explore an alternative approach to target bacteria based on acid production. Two 14 amino acid long peptides, rich in both histidine and phenylalanine residues, have been constructed based on the naturally occurring clavanin A that displays a significant increase in antimicrobial activity at low pH compared to neutral conditions. These two AMPs showed a striking pH-dependent antimicrobial activity, which correlated well with the calculated charge distribution (74).

2.3. Vaccines

Beside the AMPs produced by the innate immune system, another line of defense in human body that can be utilized against *S. mutans* colonization is the specific antibody production from adaptive immunity. Immune defense in dental caries is mediated mainly by secretory IgA (sIgA) antibodies present in saliva and generated by the mucosal immune system (75). Mucosal immunization with *S. mutans* antigens at inductive sites, including gut-associated lymphoid tissue (GALT) and nasopharynx-associated lymphoid tissue (NALT), results in the migration of antigen-specific IgA-producing B cells to effector organs, such as the salivary glands. This is followed by the differentiation and maturation of these B cells and the secretion of IgA in the lamina propria, where it crosses the effector tissue ducts into the saliva (76).

In the view of vaccine development, research focus is mainly on the incorporation of purified bacterial antigens into mucosal immune systems and delivery to mucosal IgA-inductive sites. The three main types of *S. mutans* antigen that are involved in dental-caries pathogenesis and for which specific secretory IgAs have been found are antigen I/II, GTFs and glucan-binding proteins (GBP) (77). Accordingly, the mechanisms of action of these specific antibodies are: clearance of bacteria in saliva by antibody-mediated aggregation, inhibition of the adherence of bacteria by blocking bacterial-surface receptors, and modification of metabolically functions of bacterial enzymes (77,78).

Antigen I/II is the surface adhesin of *S. mutans*. It contains an alanine-rich tandem-repeating region and a proline rich repeat region, which are associated with the adhesion activity (79). Secretory IgA specific for intact antigen I/II or its binding domain is able to inhibit adhesion of *S. mutans* to saliva-coated hydroxyapatite (80) and their colonization on tooth (81). The effect of antigen I/II vaccine or monoclonal antibody of this antigen is tested both in animal models and in human clinical study (82,83). Results showed that vaccine or monoclonal antibody can effectively prevent *S. mutans* colonization. However, more clinical data regarding to caries experience are required before they can be proved effective against dental caries. New peptide subunits of *S. mutans* antigen I/II have been disclosed by

identifying *S. mutans*' T-cell and B-cell epitopes. These peptides are able to adhere to a mammalian tooth in a competitive manner with naturally occurring *S. mutans* antigen I/II, thus preventing or diminishing the adhesion of *S. mutans* to the tooth and also stimulate a T-cell and B-cell response against *S. mutans* (84). To overcome potential disadvantages, including unwanted antibody response and allergy reactions, associated with non-human source monoclonal antibodies, an isolated diabody composed of a human heavy chain variable domain and a human light chain variable domain which specifically binds *S. mutans* antigen I/II has been developed (85). This diabody derivative is capable of aggregating *S. mutans* cells, making it a useful candidate therapeutic agent for passive immunization against dental caries.

GTFs are important in synthesis of water-soluble and water-insoluble glucans. Its activity is mediated through both catalytic and glucan-binding domains, antibody binding to any GTF activity domain can inactivate GTFs and interfere with synthesis of glucans, inhibiting accumulation of (77). Vaccines derived from either one of these two domains can induce antibody production and prevent caries formation by inhibit *S. mutans* GTFs (86). Interestingly, the immunogenicity of the vaccine is significantly improved if the vaccine is a diepitopic vaccine that containing peptide epitopes from both domains (87). Smith, et al. disclosed synthetic vaccine compositions and immunogenic compositions which are GTF subunit vaccines containing one or more epitopes for prevention of dental caries (88,89).

GBPs are a group of glucan receptors that participate in the formation of dental biofilms and the accumulation of *S. mutans*. Among the three GBPs found in *S. mutans* (GBP-A, B and C), only GBP-B has been show to induce a protective immune response to experimental dental caries (90). However, it has been found that using chimeric polypeptide containing both a fragment of a GBP-B polypeptide and a GTF polypeptide can elicit a significantly higher antibody titer than using GBP-B alone (91,92).

Except for vaccines and monoclonal antibodies developed from the aforementioned antigens, other antigens from *S. mutans* have also been utilized to develop vaccines. *S. mutans* and *S. sobrinus* produce extracellular proteins, virulence-associated immunomodulatory extracellular proteins (VIP), which provide the suppression of the immune response in the host through the early production of IL-10. A vaccine is developed from active VIP that can induce the immunoneutralization of the VIP immunomodulatory effects therefore prevent dental caries (93). In another invention, novel vaccines based on the extracellular glucan component of the cariogenic plaque are developed (94). The antigenicity of the glucan may be enhanced by covalently coupling the glucan to one or more moieties, including at least one T cell-dependent antigen (e.g. GTF and/or haptens), to form a conjugate vaccine.

2.4. Probiotics and Replacement Therapy

As mentioned above, treatment measures that aimed at wiping out the entire oral flora might lead to unwanted negative consequences. Therefore, more and more efforts in caries research are devoted in looking for approached that can selectively inhibit oral pathogens rather than the whole microbial community. Probiotics as defined by the World Health Organization are live micro-organisms, which, when administered in adequate amounts, confer a health benefit on the host (43). The mechanism of probiotic action might be the disruption of plaque biofilm formation through competition for binding sites on host tissues and other bacteria, and competition for nutrients; or the production of antimicrobial compounds that inhibit oral bacteria, such as a organic acids, hydrogen peroxide, low-molecular-weight antimicrobial compounds, bacteriocins and adhesion inhibitors produced by lactic acid bacteria (43,95).

Inspired by the success in using probiotics to control gastro-intestinal diseases, some of the gastrointestinal bacteria strains have been tested in experimental studies and clinical trials to control the growth of cariogenic bacteria. For example, long-term consumption of milk containing *Lactobacillus rhamuosus* GG strain can reduce initial caries in kindergarten children (96). Ingestion of *Lactobacillus reuteri* ATCC 55739 (97) or *Bifidobacterium* DN-173 010 (98) can induce significant reduction of cariogenic *S. mutans* in saliva. Mollstam, et al. (99) disclosed several new strains of *Lactobacillus*, including *L. reuteri* CF2-7F (ATCC PTA-4965), *L. reuteri* MF2-3 (ATCC PTA-4964) and especially *L. reuteri* FJ1 “Prodentis” (ATCC PTA-5289) and *L. reuteri* FJ3 (ATCC PTA-5290), that have good antimicrobial activity against *S. mutans* and good binding characteristics to oral mucin and thereby prevent, reduce or treat dental caries. Due to the existence of mutual antagonistic effects among various oral streptococci, implantation of specific oral streptococci or the encouragement of their growth in dental plaque may thus be considered a probiotic approach by encouraging an ecological shift (100). Chilcott, et al. (101) disclosed novel *Streptococcus salivarius* strains that produce bacteriocin-like inhibitory substances with a broad spectrum of activity against dental caries causing organisms including *S. mutans*. Also, *Streptococcus oligofermentans*, a bacterium that could be isolated only from caries-free humans, was found to metabolize lactic acid into hydrogen peroxide, thus inhibiting the growth of *S. mutans* (102).

In addition to probiotics, another measure that competitively reduces the pathogen composition in the oral flora has emerged with the advances in gene engineering and DNA recombination technology. This method is the so-called replacement therapy. Several mutated strains of *S. mutans* that lack the machinery to efficiently metabolize fermentable carbohydrates to organic acids have been developed. In one case, a non-acid-producing *S. mutans* strain BCS3-L1 that produces an antibiotic called mutacin 1140 active against other *S. mutans* strains to replace the naturally occurring cariogenic strains in oral cavity has been developed (103-105). This strain was significantly less cariogenic than the parent strain JH1140 due to the delete of lactic acid dehydrogenase open reading frame. The clone was also shown to be genetically stable which did not revert to producing acid in both *in vivo* and *in vitro* test systems and is currently awaiting evaluation for its efficacy in humans (106,107). In another study, the ability of *S. mutans* to produce extracellular glucans is blocked in a mutation by deleting the GTF-C gene. Introducing this new strain into an *in vitro* mixed biofilm model has resulted in a decrease in extracellular matrix component from 51 to 33 percent of the biofilm volume (108).

2.5. Sugar Substitutes

The formation of dental caries and the demineralization of tooth enamel are not caused by pathogenic bacteria itself, but most directly caused by the acids produced by acidogenic bacteria. Dietary sugars such as sucrose glucose and lactose can be ferment by MS such as *S. mutans* and *S. sobrinus* to produce lactic acid which is most important and the strongest acid produced in large quantities that involved in the etiology of dental caries (21). During the past 50 years, several clinical studies (109-111) as well as laboratory studies (112,113) have clearly demonstrated that sugar intake plays a major role in the initiation and progression of dental caries. Data collected from *in vivo* and *in vitro* studies indicate that sugar substitutes exhibit potential anti-caries effect in several aspects including (114): inhibition of insoluble glucan synthesis from sucrose; decrease in MS numbers in whole saliva and plaque; increase in the buffering capacity and pH of dental plaque; and interference with enamel demineralization and an increase in enamel remineralization.

One of the most extensively studied sugar substitute is Xylitol. Xylitol is a five carbon sugar alcohol (Polyol) that looks and tastes like sucrose. Xylitol can not be utilized and fermented by MS or other microorganisms in oral cavity (115), but it can be directly absorbed by

human small intestine and subsequently metabolized (114). Both *in vitro* and *in vivo* studies (116,117) showed that the plaque pH is not affected by the intake of xylitol, and it was found that xylitol has a bacteriostatic effect on *S. mutans* (118) by creating a futile cycle that consumes cellular ATP. In a futile cycle, Xylitol is transported across bacteria cell membrane by a phospho-transferase system, generating xylitol-5-phosphate which can not be metabolized and may subsequently be dephosphorylated and exported at the expense of ribitol-5-phosphate (119,120). Numerous clinical studies have shown that consumption of xylitol can significantly reduce saliva and plaque *S. mutans* levels (121,122), probability of mother-child transmission of MS (123,124), and dental caries increment (111,125). These results also indicated that the caries reduction effect of Xylitol is dose and frequency dependent (126), and a plateau effect might occur with higher dosages (127). However, it should be noticed that even though Xylitol chewing-gum seems to be a promising measure for dental caries prevention, a dose-response relationship is still in urgent need for the establishment of a Xylitol based therapy against dental caries, especially when there are cases where Xylitol failed to show caries reduction effect at lower doses (128,129). Further, most of these clinical studies have chosen chewing gums as the carrier for Xylitol, and since frequent chewing is also proven to protect against caries by stimulating saliva flow (130), proper control groups such as sugar-free gum group but not no-gum-chewing group should be used to rule out other factors that might affect caries experience.

Another common sugar substitute is Sorbitol, which is a six carbon sugar alcohol. Sorbitol is the sugar alcohol most frequently added to food, both in solid and in liquid form (114). It is cheaper than xylitol but its sweetness is only 50 per cent that of sucrose or xylitol (21). Sorbitol is metabolized in the same manner as Xylitol, however, the major difference between Sorbitol and Xylitol is that Sorbitol can be fermented by MS. Although acid production can occur during Sorbitiol metabolism by the bacteria, the rate is significantly slower than that of other dietary sugars such as sucrose, glucose and fructose (131), therefore Sorbitol is considered non-cariogenic (132). A recently published review article statistically analyzed the clinical studies of polyol-containing chewing gums and suggested that the caries prevention effect of Sorbitol might be weaker than that of Xylitol (133).

Even though there are many oral hygiene product containing sugar substitute on the market, their major role is still a non-cariogenic sweetener. The anti-caries therapeutic effect of sugar substitute is under much debate (134) especially when the dose-effect relationship still have not established yet as mentioned above. Some work has turned to the idea of combining sugar substitute with other therapeutic agents. Takatsuka, et al. (135) disclosed an oral composition comprising sugar substitute palatinit which exerts a synergistic effect when combined with a fluorine or zinc compound. It is found that palatinit can enhance the remineralization, and, further, that the inclusion of palatinit in a combination with a fluorine or zinc compound can enhance the remineralization due to the synergistic effect of both ingredients. Kaufmann, et al. (136) disclosed a hard candy composition containing sugar substitute with an ammonium salt and may further containing an alkaline metal salt and an alkaline earth metal salt. Sugar substitute is included in the composition to reduce demineralization while the alkaline compounds creating a pH value that promotes remineralization of the tooth enamel by neutralizing plaque acids.

The antibacterial properties of sugar substitutes are rather weak when compare to other therapeutic antimicrobial agent, therefore, prolonged exposure of these sugar substitutes in the oral cavity on a daily bases is required for them to be effective (133). Traditional delivering vehicles such as chewing-gums, hard candies and mints can only provide contact of the sugar substitutes with tooth surface for a few minute or even seconds. Therefore, novel delivery vehicles are still needed for the effective delivery of sugar substitutes before they can be considered as therapeutically effective.

3. Therapeutics to promote the remineralization process

Different from the demineralization process, which is related to several biological and physiological factors such as bacterial biofilm, AMPs and antibody production from the innate and adaptive immune system, remineralization process is straightly an inorganic chemistry process. In this process, the recrystallizing of calcium and phosphate (primarily from saliva) in the water among the enamel or dentin crystals to the surfaces of existing crystal remnants is time consuming. However, the mineral formed during remineralization is more resistant to acid than the original enamel or dentin mineral, especially if fluoride is present and incorporated into the new crystal surfaces (3). The importance of remineralization can be illustrated through the dramatic downturn in the numbers of decayed, missing, and filled teeth from the 1970s, and the increase in the percent of the population that is apparently clinically caries-free (137). The major factor of this triumph in caries prevention is attributed to the use of fluoride-containing toothpastes which enhanced the remineralization process (138). Therefore, therapeutics that can improve the remineralization process are just as important as therapeutics agents that prevent the demineralization caused by dental biofilm.

3.1. Fluoride

Fluoride therapy has been the main caries preventive strategy since the introduction of water fluoridation schemes several decades ago (139). Fluoride salts can be commonly found in drinking water, toothpastes and mouse rinses which are available to majority of the US population. However, it has been pointed out that, even with such a high availability, an increase in caries-free population reached a plateau in 1990s, and there were still at least 60% of teenagers around that time, and most likely still today, had observable dental decay. Thus the anti-caries effect of traditional fluoride therapy is still limited (137). Several *in vitro* studies on a pH cycling model, which consists of alternating periods of demineralization and remineralization in the laboratory over a three-week period, indicated a linear relationship between the logarithm of the fluoride level and the net amount of remineralization. A rapid rise in effect was observed as the fluoride concentration increased from 0.03 ppm to 0.1 ppm (140,141). These findings are also supported by a recent report showed that a 70% reduction in caries versus control for highrisk children who wore a fluoride-releasing glass device in their mouths. The mean salivary fluoride levels were 0.11 ppm in the test group versus 0.03 ppm in the control group over a two-year period (142). However, baseline fluoride levels in saliva are known to be around 0.02 ppm or less, dependent on the fluoride level in drinking water and the use of fluoride products (143). Current delivery measure such as toothpastes, mouse rinses and even varnish (144) are unable to maintain this concentration, thus is not adequate for high caries challenge (145). Therefore, slow-release fluoride devices have attracted a lot of attention lately due to its ability to maintain a therapeutic concentration of fluoride in the oral cavity for an extended period of time.

The slow-release fluoride device normally involves the attachment of a small fluoride-containing device (in the dimension of mm) to the crown of a tooth (usually the buccal surface of a upper molar) and slowly release fluoride over a prolonged period of time (at least a year). The copolymer membrane device was developed in the 1970s and was designed to be attached on or near the tooth surface. This system was designed as a membrane-controlled reservoir-type with an inner core of hydroxyethyl methacrylate (HEMA) / methyl methacrylate (MMA) copolymer (50:50 mixture) which is surrounded by a 30:70 HEMA/MMA copolymer membrane that controls the rate of fluoride release from the core through a saturated fluoride concentration difference between the core and the outer membrane (146). A 6-month clinical study using this device showed a significant increase in salivary fluoride concentration from a baseline mean of 0.07-0.69 mg/mL on day 14 post-

insertion. The device retention was 85% after 6 months and, of the devices retained, 100% were functional (147). A similar delivery device, the so called “glass device” which dissolves slowly when moist in saliva, releasing fluoride without significantly affecting the device's integrity (146). A recent 6-month clinical study in UK showed that mean salivary fluoride levels in the child volunteers using this device was 0.17±0.1 ppm fluoride compared with 0.025 ppm baseline levels, and the retention rate of the devices was 93% (148).

Traditional silicate glasses from which fluoride can be slowly leached generally exhibit low fluoride retention (loading) capability in the device, and glasses formed by phosphate are easily attacked by water. Algar, et al. (149) recently disclosed a glass composition that increase the fluoride loading and also release fluoride at a stable rate. The composition was formed by combining and melting a phosphorus oxide and at least one of sodium, potassium, lithium or aluminum in oxide and/or fluoride form. The glass composition optionally comprising silicon in an amount up to about 5% by weight allowing a therapeutically effective amount of fluoride to be slowly released over time. To increase the retention rate and prolong retention time of the slow-releasing device, Jessop, et al. (150,151) disclosed a dental bracket and associated kit used to attach a fluoride-releasing pellet on a tooth. The pellet is placed in and attached to the dental bracket through adhesive resin. The bracket binds to the patient's tooth using a curable adhesive resin that is cured by at least one of chemical curing, light curing or air curing. The pellet is designed to slowly release fluoride and may be replaced every 6 months to 2 years. Replacement of the fluoride-releasing pellet may be performed at home or at a dentist's office. The bracket can be attached to a patient's tooth for up to 20 years.

A dicalcium phosphate anhydrous (DCPA) nanocomposite was developed by Xu, et al. as a restoration material that can slowly release high levels of $CaPO_4$ requisite for remineralization (152,153). In a recent report, they incorporate novel CaF_2 nanoparticles to develop stress-bearing, F-releasing nanocomposite. This nanocomposite can slowly release Fluoride ions for more than 10 weeks at a release rate higher than traditional and resin-modified glass ionomer materials (154). Another way to prolong the fluoride releasing capability is to periodically recharge the releasing device. Xu, et al. (155) provides a class of polymerizable monomers containing chelating groups and fluoride-exchanging metal chelates that can release fluoride into an aqueous solution, and that can "recharge" by taking up fluoride from an aqueous solution containing a high concentration of fluoride (e.g., a fluoridated toothpaste or mouthwash). These compositions may be incorporated into dental composite restorative materials or other dental materials, to produce materials with high fluoride release rates, and high fluoride recharge capability. Other measures such as glass ionomer cements (156) and tissue scaffolds (157) were also developed to increase fluoride retention in oral cavity, but they are used more often in caries restoration rather than prevention, and will not be discussed in details in this review.

In addition to increase fluoride retention in the oral cavity, approaches to enhance the remineralization effect of fluoride or reduce the requirement of fluoride level in the oral cavity have also been developed. Recently, Faller, et al. (158) disclosed novel methods of enhancing fluoride incorporation into tooth and mineralization of tooth by use of specialized phosphonate containing polymers or telomers. Due to the polyphosphate nature of these polymers, they are able to provide desired surface conditioning effects including: 1) the effective desorption of undesirable adsorbed pellicle proteins, in particular those associated with undesirable microbial species; 2) creating a hydrophilic tooth surface immediately after treatment; and 3) maintaining surface conditioning effects and control of pellicle film for extended periods following product use. The improvement of surface properties eventually lead to increased fluoride deposition and remineralization of tooth. As mentioned earlier, tooth enamel crystals (hydroxyapatite) have “weak spots” that are less resistant to

dissolution by acids. The treatment of tooth enamel by carbon dioxide laser (or other source) irradiation or by high temperatures remove or vaporize carbonate sites. At the same time, laser treatment also causes shrinkage of the hydroxyapatite crystal, which will require less fluoride to provide protection. An improved novel laser treatment method has been disclosed by Cozean, et al. (159) which makes the tooth more resistant to acid and easier to bound fluoride, thus requiring a lower concentration of fluoride. The effect was found to be synergistic.

Even though fluoride has the ability to inhibit demineralization and plaque bacteria, the major role of fluoride is still enhancement of remineralization. Therefore, to prevent dental caries (especially when against high caries challenges) with only fluorides is unrealistic (137). Factors that can reduce the demineralization burden are also beneficial and should be considered in fluoride therapy. Recently, two clinical studies have shown that Colgate Total Toothpaste which contain 0.3% triclosan, 2.0% polyvinylmethyl ether/maleic acid (PVM/MA) copolymer, and 0.243% sodium fluoride in a silica base is statistically significantly better in controlling dental plaque when compared to a control dentifrice which contain only 0.243% sodium fluoride in a silica base for a 12h (160) or 24h (161) period of time. The results of a three-year clinical trial of root caries and dental crowns among adults showed a statistically significant difference for root caries and dental crown failure scores, both favoring the Colgate Total Toothpaste when compare to a control toothpaste which contain only sodium fluoride (162). However, another three-year clinical study did not show differences in plaque scores between Colgate Total Toothpaste group and standard sodium fluoride toothpaste group among periodontitis-susceptible subjects (163). More work have to be done to evaluate the remineralization efficacy of fluoride when used in combination with anti-plaque agents, as well as to develop more delivery systems that could combine anti-plaque therapy and fluoride therapy together to achieve a synergistic effect.

3.2. Casein Phosphopeptides

Dairy products such as milk, milk concentrates and cheese are recognized as non-cariogenic or cariostatic in several laboratory studies due to the presence of milk phosphoprotein, casein (164). The casein phosphopeptides (CPP) are derived from casein by tryptic digestion. In 1987, Reynolds found that CPPs were incorporated into the intra-oral appliance plaque and were associated with a substantial increase in the plaque's content of calcium and phosphate (165). All CPPs contain the sequence motif -Pse-Pse-Pse-Glu-Glu-, where Pse is a phosphoseryl residue. Through these multiple phosphoseryl residues, CPPs have a marked ability to stabilize calcium phosphate ions in solution and to form a amorphous calcium phosphate (ACP) complex, referred to as CPP-ACP (164,166). It is proposed that the CPP bind to the forming alkaline ACP nanoclusters, producing a metastable solution thereby preventing ACP growth to the critical size required for nucleation and precipitation (167). CPP-ACP binds strongly to hydroxyapatite and can diffuse and retained in dental plaque, therefore is able to buffer acid and substantially raise the level of calcium phosphate in plaque or close proximity to the tooth surface, and thus inhibits enamel demineralization and enhances remineralization (165). Stable and highly soluble CPP-ACP has been trademarked as Recaldent™ and has now been commercialized in sugar-free gum and mints and in dental professional products (Tooth Mousse™) (168).

Several randomized clinical trials (RCT) have shown that CPP-ACP added to sugar-free chewing gums (169), tooth paste (170) or dental cream (171,172) increased enamel subsurface remineralization. These RCT results suggested both a short-term remineralization effect of CPP-ACP and a caries-preventing effect for long-term clinical CPP-ACP use (173). Besides ACP, CPP also stabilize calcium fluoride phosphate (ACFP) and forming CPP-ACFP (174). In this case, calcium and phosphate ions co-localize at the tooth surface with fluoride ion, therefore increases the degree of saturation with respect to fluorapatite and

promoting remineralization of enamel with fluorapatite. This enhanced remineralization effect is demonstrated in an *in vitro* study (168).

4. Expert Opinion

For antimicrobial therapies aimed at inhibiting demineralization, a clear trend in the literature is to find anti-plaque therapeutics from natural sources, including natural antimicrobials from plant extract, derivatives from natural AMPs, and use natural replacement strains or natural immune system. However, traditional antimicrobials, such as chlorhexidine or triclosan, are still the most cost-effective therapeutic agents available to the general population. In future studies, more attention should be place on developing measures such as targeting or controlled release mechanisms for these drugs in order to reduce the unwanted effect including the development of drug resistance strain and undifferentiated killing. For remineralization therapies, the challenge is obviously our sole dependence upon fluoride therapy. Many clinical studies have point out that fluoride therapy alone is not enough to overcome high caries challenges. It is probably the right time find additional and more effective remineralization measures as alternatives to fluoride therapy.

For both demineralization prevention and remineralization promotion, one vital concept that may predict the success of the therapy is effective drug retention on tooth surface. Without retention, the reduced pathogen level by antimicrobial agent in the oral cavity can be re-established between two doses, and the remineralized enamel crystal surface by a short exposure to remineralization agent will be overwhelmed by the constant demineralization process. Novel drug delivery systems should be designed to fit the needs for enhanced retention and controlled release on tooth surface. Drug delivery systems that have been designed for bone-targeted delivery may be adapted to provide retention mechanisms on tooth surface. On-demand release property and bacterium-specific delivery can also be achieved by further modification of the delivery system with pH-sensitive materials and special targeting moieties.

Therefore, we believe both discovery of new anti-caries agents and the development of dentotropic delivery systems for these agents are important for the prevention and treatment of dental caries. Future research in this field will probably lies within these two directions.

Article Highlights

- Therapeutics against dental caries can be generally divided into two categories: demineralization inhibitors and remineralization promoters.
- Chemoprophylactic agents are effective against dental biofilm, but their retention in oral cavity need to be improved.
- Antimicrobial peptides are potential replacement of traditional antibiotics to prevent the creation of antibiotic-resistant organisms.
- Vaccines and monoclonal antibodies can be utilized against *S. mutans* colonization; however, more clinical studies are needed to prove their effectiveness against caries formation.
- Probiotics and replacement therapy can reduce the pathogen composition in the oral flora by selectively inhibit cariogenic bacteria rather than the whole microbial community.
- Sugar substitutes are non-cariogenic and chewing gums containing sugar substitutes can reduce caries experience. A dose-response relationship is urgently needed to establish sugar substitutes as a therapy against dental caries.

- Fluoride is the most available and commonly used remineralization agent. However, many clinical studies have point out that fluoride therapy alone is not enough to overcome high caries challenges. Its retention in oral cavity is also another challenge.
- Casein phosphopeptides (CPP) is another reminerialization agent derived from milk phosphoprotein, casein. Recent clinical trails suggested both a short-term remineralization effect and a caries-preventing effect for long-term clinical CPP-amorphous calcium phosphate (ACP) use.

This box summarises key points contained in the article.

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Declaration of interest

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References

1. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet Jan 6;2007 369(9555):51–9. [PubMed: 17208642]
2. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc Jul;2000 131(7): 887–99. [PubMed: 10916327]
3. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol Feb;1999 27(1):31–40. [PubMed: 10086924]
4. Garcia-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: the role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. J Am Dent Assoc May;2008 139(Suppl):25S–34S. [PubMed: 18460677]
5. Fejerskov, O.; Kidd, E., editors. Dental caries: the disease and its clinical management. Blackwell Monksgaard; Copenhagen, Denmark: 2003.
6. Marsh, P.; Martin, MV. Oral Microbiology. 4th edn.. Wright; Oxford: 1999.
7. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004;83(Spec No C:C35-8)
8. Featherstone JD. The continuum of dental caries--evidence for a dynamic disease process. J Dent Res 2004;83(Spec No C:C39-42)
9. Featherstone JD. Dental caries: a dynamic disease process. Aust Dent J Sep;2008 53(3):286–91. [PubMed: 18782377]
10. Seow WK. Biological mechanisms of early childhood caries. Community Dent Oral Epidemiol 1998;26(1 Suppl):8–27. [PubMed: 9671196]
11. ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med 1991;2(3):283–96. [PubMed: 1892991]
12. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol Sep;1995 15(3):169–75. [PubMed: 8519474]
13. Scheie AA, Petersen FC. The Biofilm Concept: Consequences for Future Prophylaxis of Oral Diseases? Crit Rev Oral Biol Med 2004;15(1):4–12. [PubMed: 14761896]
14. Zander HA, Bibby BG. Penicillin and caries activity. J Dent Res Oct;1947 26(5):365–8. [PubMed: 18917755]
15. Jordan HV, De Paola PF. Effect of a topically applied 3 percent vancomycin gel on Streptococcus mutans on different tooth surfaces. J Dent Res Jan-Feb;1974 53(1):115–20. [PubMed: 4520429]
16. Anderson MH. A review of the efficacy of chlorhexidine on dental caries and the caries infection. J Calif Dent Assoc Mar;2003 31(3):211–4. [PubMed: 12693819]

17. Duckworth RM. The science behind caries prevention. Int Dent J Dec;1993 43(6 Suppl 1):529–39. [PubMed: 8138324]
18. Walker C. Effects of sanguinarine and Sanguinaria extract on the microbiota associated with the oral cavity. J Can Dent Assoc 1990;56(7 Suppl):13–30. [PubMed: 2207850]
19. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res Mar;1994 73(3):682–91. [PubMed: 8163738]
20. Marshand PD, Bradshaw DJ. Microbiological effects of new agents in dentifrices for plaque control. Int Dent J 1993;43:399–406. [PubMed: 8282422]
21. Balakrishnan M, Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. Aust Dent J Dec;2000 45(4):235–45. [PubMed: 11225524]
22. Guggenheim B, Giertsen E, Schupbach P, Shapiro S. Validation of an in vitro biofilm model of supragingival plaque. J Dent Res Jan;2001 80(1):363–70. [PubMed: 11269730]
23. Shapiro S, Giertsen E, Guggenheim B. An in vitro oral biofilm model for comparing the efficacy of antimicrobial mouthrinses. Caries Res Mar-Apr;2002 36(2):93–100. [PubMed: 12037365]
24. Moshrefi A. Chlorhexidine. J West Soc Periodontol Periodontal Abstr 2002;50(1):5–9. [PubMed: 12049062]
25. Yates R, Jenkins S, Newcombe R, et al. A 6-month home usage trial of a 1% chlorhexidine toothpaste (1). Effects on plaque, gingivitis, calculus and toothstaining. J Clin Periodontol Feb; 1993 20(2):130–8. [PubMed: 8436632]
26. Barton, S.; Galley, E. Oral hygiene composition. US5695745. 1997.
27. Furia TE, Schenkel AG. A new, broad spectrum bacteriostat. Soap Chem Specialties 1968;44:47–50. 116–22.
28. Stephen KW, Saxton CA, Jones CL, et al. Control of gingivitis and calculus by a dentifrice containing a zinc salt and triclosan. J Periodontol Nov;1990 61(11):674–9. [PubMed: 2254833]
29. Svatun B, Sadxton CA, Huntington E, Cummins D. The effects of three silica dentifrices containing Triclosan on supragingival plaque and calculus formation and on gingivitis. Int Dent J Aug;1993 43(4 Suppl 1):441–52. [PubMed: 8282427]
30. Raghavan SL, Schuessel K, Davis A, Hadgraft J. Formation and stabilisation of triclosan colloidal suspensions using supersaturated systems. Int J Pharm Aug 11;2003 261(1-2):153–8. [PubMed: 12878404]
31. Gilbert RJ, Williams PE. The oral retention and antiplaque efficacy of triclosan in human volunteers. Br J Clin Pharmacol May;1987 23(5):579–83. [PubMed: 3593627]
32. Barabolak, RM.; Witkewitz, DL. Anti-plaque emulsions and products containing same. US7022314. 2006.
33. Nelson, DGA.; Sheehan, CJ. Cyclodextrins in dental products. US6942848. 2005.
34. Gaffar, A.; Nabi, N.; Afflitto, J. Process for inhibiting S. mutans and caries. US6136298. 2000.
35. Iyer, LM.; Barkans, DE.; Hench, BD. Gums containing antimicrobial agents. US6248309. 2001.
36. Nabi N, Mukerjee C, Schmid R, Gaffar A. In vitro and in vivo studies on triclosan/PVM/MA copolymer/NaF combination as an anti-plaque agent. Am J Dent Sep;1989 2(Spec No: 197-206)Nabi N, Mukerjee C, Schmid R, Gaffar A. In vitro and in vivo studies on triclosan/PVM/MA copolymer/NaF combination as an anti-plaque agent. Am J Dent 1989;2(Spec No: 197-206)
37. Nabi, N.; Gaffar, A. Antibacterial antiplaque oral composition. US4894220. 1990.
38. Gaffar A, Afflitto J, Nabi N. Chemical agents for the control of plaque and plaque microflora: an overview. Eur J Oral Sci Oct;1997 105(5 Pt 2):502–7. [PubMed: 9395116]
39. Gaffar A, Nabi N, Kashuba B, et al. Antiplaque effects of dentifrices containing triclosan/copolymer/NaF system versus triclosan dentifrices without the copolymer. Am J Dent Sep;1990 3(Spec No:S7-14)
40. Gaffar A, Afflitto J, Nabi N, et al. Recent advances in plaque, gingivitis, tartar and caries prevention technology. Int Dent J Feb;1994 44(1 Suppl 1):63–70. [PubMed: 8021038]
41. Plochocka, K. Polymeric delivery and release systems for oral care actives. US6464961. 2002.
42. Chen F, Liu XM, Rice KC, et al. Tooth-binding micelles for dental caries prevention. Antimicrob Agents Chemother Nov;2009 53(11):4898–902. [PubMed: 19704121]

43. Allaker RP, Douglas CW. Novel anti-microbial therapies for dental plaque-related diseases. Int J Antimicrob Agents Jan;2009 33(1):8–13. [PubMed: 18804350]
44. Darout IA, Albandar JM, Skaug N, Ali RW. Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. J Clin Periodontol May;2002 29(5):411–20. [PubMed: 12060423]
45. Takarada K, Kimizuka R, Takahashi N, et al. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol Feb;2004 19(1):61–4. [PubMed: 14678476]
46. Hamilton-Miller JM. Anti-cariogenic properties of tea (Camellia sinensis). J Med Microbiol Apr; 2001 50(4):299–302. [PubMed: 11289514]
47. Hirasawa M, Takada K, Otake S. Inhibition of acid production in dental plaque bacteria by green tea catechins. Caries Res 2006;40(3):265–70. [PubMed: 16707877]
48. Fine DH, Furgang D, Lieb R, et al. Effects of sublethal exposure to an antiseptic mouthrinse on representative plaque bacteria. J Clin Periodontol May;1996 23(5):444–51. [PubMed: 8783049]
49. Petti S, Scully C. Polyphenols, oral health and disease: A review. J Dent Jun;2009 37(6):413–23. [PubMed: 19303186]
50. Al-Hebshi NN, Nielsen O, Skaug N. In vitro effects of crude khat extracts on the growth, colonization, and glucosyltransferases of Streptococcus mutans. Acta Odontol Scand Jun;2005 63(3):136–42. [PubMed: 16191906]
51. Rahim ZH, Khan HB. Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of Streptococcus mutans. J Oral Sci Sep;2006 48(3): 117–23. [PubMed: 17023743]
52. Koo H, Nino de Guzman P, Schobel BD, et al. Influence of cranberry juice on glucan-mediated processes involved in Streptococcus mutans biofilm development. Caries Res 2006;40(1):20–7. [PubMed: 16352876]
53. Yu HH, Lee DH, Seo SJ, You YO. Anticariogenic properties of the extract of Cyperus rotundus. Am J Chin Med 2007;35:497–505. [PubMed: 17597508]
54. Brighenti FL, Luppens SB, Delbem AC, et al. Effect of Psidium cattleianum leaf extract on Streptococcus mutans viability, protein expression and acid production. Caries Res 2008;42(2): 148–54. [PubMed: 18367836]
55. Yu HH, Lee JS, Lee KH, et al. Saussurea lappa inhibits the growth, acid production, adhesion, and water-insoluble glucan synthesis of Streptococcus mutans. J Ethnopharmacol 2007;111:413–7. [PubMed: 17234374]
56. Papetti A, Pruzzo C, Daglia M, et al. Effect of barley coffee on the adhesive properties of oral streptococci. J Agric Food Chem Jan 24;2007 55(2):278–84. [PubMed: 17227054]
57. Yamamoto H, Ogawa T. Antimicrobial activity of perilla seed polyphenols against oral pathogenic bacteria. Biosci Biotechnol Biochem Apr;2002 66(4):921–4. [PubMed: 12036078]
58. Mezine, I.; Zhang, HZ.; Petteruti, M., et al. Oral care compositions derived from the Labiatae family. US7517541. 2009.
59. Ofek, I.; Weiss, E.; Kashman, Y., et al. Anti-microbial-adhesion fraction derived from vaccinium. US6843993. 2005.
60. Majeed, M.; Prakash, S. Composition and methods containing an antimicrobial essential oil extended from Coleus forskohlii. US6607712. 2003.
61. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science Apr 15;1994 264(5157):375–82. [PubMed: 8153624]
62. Schutze GE, Kaplan SL, Jacobs RF. Resistant Pneumococcus: a worldwide problem. Infection Jul-Aug;1994 22(4):233–7. [PubMed: 8002081]
63. Pazgier M, Hoover DM, Yang D, et al. Human beta-defensins. Cell Mol Life Sci Jun;2006 63(11): 1294–313. [PubMed: 16710608]
64. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol Mar;2005 3(3):238–50. [PubMed: 15703760]
65. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature Jan 24;2002 415(6870):389–95. [PubMed: 11807545]

66. Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol Oct;2006 6(5):468–72. [PubMed: 16890021]
67. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol Sep 1;2004 173(5):2909–12. [PubMed: 15322146]
68. Rennie J, Arnt L, Tang H, et al. Simple oligomers as antimicrobial peptide mimics. J Ind Microbiol Biotechnol Jul;2005 32(7):296–300. [PubMed: 15959729]
69. Beckloff N, Laube D, Castro T, et al. Activity of an antimicrobial peptide mimetic against planktonic and biofilm cultures of oral pathogens. Antimicrob Agents Chemother Nov;2007 51(11):4125–32. [PubMed: 17785509]
70. Reynolds, EC.; Dashper, SG.; O'Brien-Simpson, NM., et al. Antimicrobial peptides. US7588752. 2009.
71. Leung, KP.; Concannon, SP. Antimicrobial peptide and methods of use thereof. US749498. 2009.
72. Bobek, LA. D-isomers of antimicrobial peptide. US7271239. 2007.
73. Eckert R, He J, Yarbrough DK, et al. Targeted killing of Streptococcus mutans by a pheromone-guided “smart” antimicrobial peptide. Antimicrob Agents Chemother Nov;2006 50(11):3651–7. [PubMed: 17060534]
74. Li L, He J, Eckert R, et al. Design and characterization of an acid-activated antimicrobial peptide. Chem Biol Drug Des 2009;75:127–32. [PubMed: 19878192]
75. Islam B, Khan SN, Khan AU. Dental caries: from infection to prevention. Med Sci Monit Nov; 2007 13(11):RA196–203. [PubMed: 17968308]
76. Youngman, KR.; Lazarus, NH.; Butcher, EC. Mucosal Immunology. 3rd ed.. Elsevier; Burlington: 2005.
77. Taubman MA, Nash DA. The scientific and public-health imperative for a vaccine against dental caries. Nat Rev Immunol Jul;2006 6(7):555–63. [PubMed: 16729013]
78. Russell MW, Hajishengallis G, Childers NK, Michalek SM. Secretory immunity in defense against cariogenic mutans streptococci. Caries Res 1999;33(1):4–15. [PubMed: 9831775]
79. Shivakumar KM, Vidya SK, Chandu GN. Dental caries vaccine. Indian J Dent Res Jan-Mar;2009 20(1):99–106. [PubMed: 19336869]
80. Hajishengallis G, Nikolova E, Russell MW. Inhibition of Streptococcus mutans adherence to saliva-coated hydroxyapatite by human secretory immunoglobulin A (S-IgA) antibodies to cell surface protein antigen I/II: reversal by IgA1 protease cleavage. Infect Immun Dec;1992 60(12): 5057–64. [PubMed: 1333448]
81. Takahashi I, Okahashi N, Matsushita K, et al. Immunogenicity and protective effect against oral colonization by Streptococcus mutans of synthetic peptides of a streptococcal surface protein antigen. J Immunol Jan 1;1991 146(1):332–6. [PubMed: 1984447]
82. Hajishengallis G, Russell MW, Michalek SM. Comparison of an adherence domain and a structural region of Streptococcus mutans antigen I/II in protective immunity against dental caries in rats after intranasal immunization. Infect Immun Apr;1998 66(4):1740–3. [PubMed: 9529105]
83. Ma JK, Hunjan M, Smith R, et al. An investigation into the mechanism of protection by local passive immunization with monoclonal antibodies against Streptococcus mutans. Infect Immun Oct;1990 58(10):3407–14. [PubMed: 2401569]
84. Lehner, T.; Kelly, C. Polypeptide fragments capable of competition with Streptococcus mutans antigen I/II. US6500433. 2002.
85. Finnern, R.; Fischer, R. Diabody which specifically binds Streptococcus surface antigen I/II and methods of use thereof. US7625561. 2009.
86. Taubman MA, Holmberg CJ, Smith DJ. Immunization of rats with synthetic peptide constructs from the glucan-binding or catalytic region of mutans streptococcal glucosyltransferase protects against dental caries. Infect Immun Aug;1995 63(8):3088–93. [PubMed: 7622235]
87. Taubman MA, Holmberg CJ, Smith DJ. Diepitopic construct of functionally and epitopically complementary peptides enhances immunogenicity, reactivity with glucosyltransferase, and protection from dental caries. Infect Immun Jul;2001 69(7):4210–6. [PubMed: 11401956]
88. Smith, DJ.; Taubman, MA. Glucosyltransferase immunogens. US7056517. 2006.

89. Smith, DJ.; Taubman, MA. Synthetic peptide vaccines for dental caries. US6827936. 2004.

90. Smith DJ, Taubman MA. Experimental immunization of rats with a Streptococcus mutans 59-kilodalton glucan-binding protein protects against dental caries. Infect Immun Aug;1996 64(8): 3069–73. [PubMed: 8757835]

91. Smith, DJ.; Taubman, MA. Immunogenicity of glucan binding protein. US7323175. 2008.

92. Smith, DJ.; Taubman, MA. Glucan binding protein and glucosyltransferase immunogens. US7163682. 2007.

93. da Conceicao Tavares Gomes, MD.; das Neves Ferreira da Silva, PM.; Rua Vilanova, MJ. Vaccine against dental caries based on virulence-associated immunomodulatory extracellular proteins produced by the cariogenic bacteria Streptococcus sobrinus and Streptococcus mutans. US7541041. 2009.

94. Lees, A.; Taubman, MA.; Smith, DJ. Conjugate vaccines for the prevention of dental caries. US7101562. 2006.

95. Meurman JH. Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci Jun; 2005 113(3):188–96. [PubMed: 15953242]

96. Nase L, Hatakka K, Savilahti E, et al. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Res 2001;35:412–20. [PubMed: 11799281]

97. Caglar E, Cildir SK, Ergeneli S, et al. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium Lactobacillus reuteri ATCC 55730 by straws or tablets. Acta Odontol Scand Oct;2006 64(5):314–8. [PubMed: 16945898]

98. Caglar E, Sandalli N, Twetman S, et al. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scand Nov;2005 63(6):317–20. [PubMed: 16512103]

99. Mollstam, B.; Connolly, E. Selection and use of lactic acid bacteria for reducing dental caries and bacteria causing dental caries. US7517681. 2009.

100. He X, Lux R, Kuramitsu HK, Anderson MH, Shi W. Achieving probiotic effects via modulating oral microbial ecology. Adv Dent Res 2009;21(1):53–6. [PubMed: 19710082]

101. Chilcott, CN.; Tagg, JR. Antimicrobial composition. US7226590. 2007.

102. Tong H, Chen W, Merritt J, Qi F, Shi W, Dong X. Streptococcus oligofermentans inhibits Streptococcus mutans through conversion of lactic acid into inhibitory H2O2: a possible counteroffensive strategy for interspecies competition. Mol Microbiol Feb;2007 63(3):872–80. [PubMed: 17302806]

103. Hillman, JD. Replacement therapy for dental caries. US5607672. 1997.

104. Hillman, JD. Lactate dehydrogenase mutants of Streptococcus mutans. US4324860. 1982.

105. Hillman, JD. Method of controlling dental caries with streptococcus mutans mutant strains. US4133875. 1979.

106. Hillman JD, Brooks TA, Michalek SM, et al. Construction and characterization of an effector strain of Streptococcus mutans for replacement therapy of dental caries. Infect Immun Feb;2000 68(2):543–9. [PubMed: 10639415]

107. Hillman JD. Genetically modified Streptococcus mutans for the prevention of dental caries. Antonie Van Leeuwenhoek Aug;2002 82(1-4):361–6. [PubMed: 12369203]

108. Thurnheer T, van der Ploeg JR, Giertsen E, Guggenheim B. Effects of Streptococcus mutans gtfC deficiency on mixed oral biofilms in vitro. Caries Res 2006;40(2):163–71. [PubMed: 16508276]

109. Gustafsson BE, Quensel CE, Lanke LS, et al. The Vipeholm dental caries study; the effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years. Acta Odontol Scand Sep;1954 11(3-4):232–64. [PubMed: 13196991]

110. Marthaler TM. Epidemiological and clinical dental findings in relation to intake of carbohydrates. Caries Res 1967;1(3):222–38. [PubMed: 4384379]

111. Kandelman D, Gagnon G. A 24-month clinical study of the incidence and progression of dental caries in relation to consumption of chewing gum containing xylitol in school preventive programs. J Dent Res Nov;1990 69(11):1771–5. [PubMed: 2229617]

112. Konig KG, Schmid P, Schmid R. An apparatus for frequency-controlled feeding of small rodents and its use in dental caries experiments. Arch Oral Biol Jan;1968 13(1):13–26. [PubMed: 5237553]
113. Grafand H, Muhlemann HR. Telemetry of plaque pH from interdental area. Helv Odontol Acta 1996;10:94–101.
114. Matsukubo T, Takazoe I. Sucrose substitutes and their role in caries prevention. Int Dent J Jun; 2006 56(3):119–30. [PubMed: 16826877]
115. Edwardsson S, Birkhed D, Mejare B. Acid production from Lycasin, maltitol, sorbitol and xylitol by oral streptococci and lactobacilli. Acta Odontol Scand 1977;35(5):257–63. [PubMed: 21508]
116. Waler SM, Rolla G. Effect of xylitol on dental plaque in vivo during carbohydrate challenge. Scand J Dent Res Aug;1983 91(4):256–9. [PubMed: 6579603]
117. Vadeboncoeur C, Trahan L, Mouton C, Mayrand D. Effect of xylitol on the growth and glycolysis of acidogenic oral bacteria. J Dent Res Aug;1983 62(8):882–4. [PubMed: 6575035]
118. Assev S, Rolla G. Further studies on the growth inhibition of Streptococcus mutans OMZ 176 by xylitol. Acta Pathol Microbiol Immunol Scand B Apr;1986 94(2):97–102. [PubMed: 3728029]
119. Hausman SZ, Thompson J, London J. Futile xylitol cycle in Lactobacillus casei. J Bacteriol Oct; 1984 160(1):211–5. [PubMed: 6090413]
120. Trahan L, Bareil M, Gauthier L, Vadeboncoeur C. Transport and phosphorylation of xylitol by a fructose phosphotransferase system in Streptococcus mutans. Caries Res 1985;19(1):53–63. [PubMed: 3856485]
121. Ly KA, Milgrom P, Roberts MC, et al. Linear response of mutans streptococci to increasing frequency of xylitol chewing gum use: a randomized controlled trial [ISRCTN43479664]. BMC Oral Health 2006;6:6. [PubMed: 16556326]
122. Milgrom P, Ly KA, Roberts MC, Rothen M, Mueller G, Yamaguchi DK. Mutans streptococci dose response to xylitol chewing gum. J Dent Res Feb;2006 85(2):177–81. [PubMed: 16434738]
123. Soderling E, Isokangas P, Pienihakkinen K, Tenovuo J. Influence of maternal xylitol consumption on acquisition of mutans streptococci by infants. J Dent Res Mar;2000 79(3):882–7. [PubMed: 10765964]
124. Thorild I, Lindau B, Twetman S. Effect of maternal use of chewing gums containing xylitol, chlorhexidine or fluoride on mutans streptococci colonization in the mothers' infant children. Oral Health Prev Dent 2003;1(1):53–7. [PubMed: 15643749]
125. Isokangas P, Alanen P, Tiekso J, Makinen KK. Xylitol chewing gum in caries prevention: a field study in children. J Am Dent Assoc Aug;1988 117(2):315–20. [PubMed: 3166474]
126. Ly KA, Milgrom P, Rothen M. Xylitol, sweeteners, and dental caries. Pediatr Dent Mar-Apr;2006 28(2):154–63. discussion 92-8. [PubMed: 16708791]
127. Ly KA, Milgrom P, Rothen M. The potential of dental-protective chewing gum in oral health interventions. J Am Dent Assoc May;2008 139(5):553–63. [PubMed: 18451371]
128. Wennerholm K, Arends J, Birkhed D, et al. Effect of xylitol and sorbitol in chewing-gums on mutans streptococci, plaque pH and mineral loss of enamel. Caries Res 1994;28(1):48–54. [PubMed: 8124697]
129. Kovari H, Pienihakkinen K, Alanen P. Use of xylitol chewing gum in daycare centers: a follow-up study in Savonlinna, Finland. Acta Odontol Scand Dec;2003 61(6):367–70. [PubMed: 14960009]
130. Twetman S. Consistent evidence to support the use of xylitol- and sorbitol-containing chewing gum to prevent dental caries. Evid Based Dent 2009;10(1):10–1. [PubMed: 19322219]
131. Hayes ML, Roberts KR. The breakdown of glucose, xylitol and other sugar alcohols by human dental plaque bacteria. Arch Oral Biol 1978;23(6):445–51. [PubMed: 30434]
132. Birkhed D, Edwardsson S, Kalfas S, Svensater G. Cariogenicity of sorbitol. Swed Dent J 1984;8(3):147–54. [PubMed: 6592773]
133. Deshpande A, Jadad AR. The impact of polyol-containing chewing gums on dental caries: a systematic review of original randomized controlled trials and observational studies. J Am Dent Assoc Dec;2008 139(12):1602–14. [PubMed: 19047666]

134. Lingstrom P, Holm AK, Mejare I, et al. Dietary factors in the prevention of dental caries: a systematic review. Acta Odontol Scand Dec;2003 61(6):331–40. [PubMed: 14960004]
135. Takatsuka, T.; Nakao, A. US7300645. 2007.
136. Kaufmann, K. Hard candy with tooth plaque-neutralizing effect comprising an ammonium salt. US5861169. 1999.
137. Featherstone JD. Remineralization, the natural caries repair process--the need for new approaches. Adv Dent Res 2009;21(1):4–7. [PubMed: 19717404]
138. Jenkins GN. Recent changes in dental caries. Br Med J (Clin Res Ed) Nov 9;1985 291(6505): 1297–8.
139. Karulf RE, Coller JA, Bartolo DC, et al. Anorectal physiology testing. A survey of availability and use. Dis Colon Rectum Jun;1991 34(6):464–8. [PubMed: 2036926]
140. Featherstone JD, Zero DT. An in situ model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization. J Dent Res Apr;1992 71(Spec No: 804-10)
141. Featherstone JD, Glena R, Shariati M, Shields CP. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. J Dent Res Feb;1990 69(Spec No:620-5) discussion 34-6.
142. Toumba KJ, Curzon ME. A clinical trial of a slow-releasing fluoride device in children. Caries Res May-Jun;2005 39(3):195–200. [PubMed: 15914981]
143. Leverett DH, Featherstone JD, Proskin HM, et al. Caries risk assessment by a cross-sectional discrimination model. J Dent Res Feb;1993 72(2):529–37. [PubMed: 8423251]
144. Eakle WS, Featherstone JD, Weintraub JA, et al. Salivary fluoride levels following application of fluoride varnish or fluoride rinse. Community Dent Oral Epidemiol Dec;2004 32(6):462–9. [PubMed: 15541162]
145. Featherstone JD. Delivery challenges for fluoride, chlorhexidine and xylitol. BMC Oral Health 2006;6(Suppl 1):S8. [PubMed: 16934125]
146. Pessan JP, Al-Ibrahim NS, Buzalaf MA, Toumba KJ. Slow-release fluoride devices: a literature review. J Appl Oral Sci Jul-Aug;2008 16(4):238–46. [PubMed: 19089254]
147. Billings RJ, Adair SM, Shields CP, Moss ME. Clinical evaluation of new designs for intraoral fluoride-releasing systems. Pediatr Dent Jan-Feb;1998 20(1):17–24. [PubMed: 9524968]
148. Andreadis GA, Toumba KJ, Curzon ME. Slow-release fluoride glass devices: in vivo fluoride release and retention of the devices in children. Eur Arch Paediatr Dent Dec;2006 7(4):258–61. [PubMed: 17164072]
149. Algar, B.; Toumba, J.; Curzon, M. Method of treating a patient's teeth using fluoride releasing glass composition. US7323160. 2008.
150. Jessop, N.; McLean, BS.; Lewis, P., et al. Dental brackets for retaining a medicament-releasing pellet on a tooth and kits including such brackets. US7097449. 2006.
151. Jessop, NT.; McLean, BS.; Fischer, DE. Fluoride-releasing pellet kit. US6997706. 2006.
152. Xu HH, Sun L, Weir MD, et al. Nano DCPA-whisker composites with high strength and Ca and PO(4) release. J Dent Res Aug;2006 85(8):722–7. [PubMed: 16861289]
153. Xu HH, Weir MD, Sun L, et al. Effects of calcium phosphate nanoparticles on Ca-PO4 composite. J Dent Res Apr;2007 86(4):378–83. [PubMed: 17384036]
154. Xu HH, Moreau JL, Sun L, Chow LC. Strength and fluoride release characteristics of a calcium fluoride based dental nanocomposite. Biomaterials Nov;2008 29(32):4261–7. [PubMed: 18708252]
155. Xu, X.; Burgess, JO.; Ding, X.; Ling, L. Fluoride-releasing compositions. US6703518. 2004.
156. Mitra, SB. Glass ionomer cement. US6765038. 2004.
157. Rutherford, B.; Somogyi, C.; White, C.; Rabins, E. Methods for treating dental conditions using tissue scaffolds. US7309232. 2007.
158. Faller, RV.; Baig, AA.; White, DJ. Method of enhancing fluoridation and mineralization of teeth. US7387774. 2008.
159. Cozean, C.; Powell, L.; Nammour, S. Method and laser apparatus for preventing tooth decay. US7163400. 2007.

160. Hu D, Zhang YP, DeVizio W, Proskin HM. A clinical investigation of the efficacy of two dentifrices for controlling oral malodor and plaque microflora overnight. J Clin Dent 2008;19:106–10. [PubMed: 19301517]

161. Barnes VM, Richter R, Vandeven M, et al. Clinical investigation of the antiplaque efficacy of a new variant of a commercially available triclosan/copolymer/fluoride dentifrice. J Clin Dent 2008;19(3):81–4. [PubMed: 19301513]

162. Vered Y, Zini A, Mann J, et al. Comparison of a dentifrice containing 0.243% sodium fluoride, 0.3% triclosan, and 2.0% copolymer in a silica base, and a dentifrice containing 0.243% sodium fluoride in a silica base: a three-year clinical trial of root caries and dental crowns among adults. J Clin Dent 2009;20(2):62–5. [PubMed: 19591339]

163. Matthews DC. Powered toothbrush plus triclosan only as effective as manual brush and fluoride toothpaste for periodontal maintenance patients. Evid Based Dent 2008;9(3):74–5. [PubMed: 18927564]

164. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist Jan-Feb;1998 18(1):8–16. [PubMed: 9791302]

165. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. J Dent Res Jun;1987 66(6):1120–7. [PubMed: 3476583]

166. Reynolds, E. Calcium phosphopeptide complexes. US7312193. 2007.

167. Bergt C, Fu X, Huq NP, et al. Lysine residues direct the chlorination of tyrosines in YXXK motifs of apolipoprotein A-I when hypochlorous acid oxidizes high density lipoprotein. J Biol Chem Feb 27;2004 279(9):7856–66. [PubMed: 14660678]

168. Cross KJ, Huq NL, Reynolds EC. Casein phosphopeptides in oral health--chemistry and clinical applications. Curr Pharm Des 2007;13(8):793–800. [PubMed: 17430181]

169. Morgan MV, Adams GG, Bailey DL, et al. The anticariogenic effect of sugar-free gum containing CPP-ACP nanocomplexes on approximal caries determined using digital bitewing radiography. Caries Res 2008;42(3):171–84. [PubMed: 18446025]

170. Rao SK, Bhat GS, Aradhya S, et al. Study of the efficacy of toothpaste containing casein phosphopeptide in the prevention of dental caries: a randomized controlled trial in 12- to 15-year-old high caries risk children in bangalore, India. Caries Res 2009;43(6):430–5. [PubMed: 19864905]

171. Andersson A, Skold-Larsson K, Hallgren A, et al. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. Oral Health Prev Dent 2007;5(3):229–33. [PubMed: 17977295]

172. Bailey DL, Adams GG, Tsao CE, et al. Regression of post-orthodontic lesions by a remineralizing cream. J Dent Res Dec;2009 88(12):1148–53. [PubMed: 19887683]

173. Yengopal V, Mickenautsch S. Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis. Acta Odontol Scand Aug 21;2009 :1–12. [PubMed: 19701818]

174. Reynolds, E. Calcium phosphopeptide complexes. US7312193. 2007.