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Reducing dental plaque formation and caries development. A review of current methods and implications for novel pharmaceuticals

Povilas Kalesinskas, Tomas Kačergius, Arvydas Ambrozaitis, Vytautė Pečiulienė, Dan Ericson

SUMMARY

Dental caries is an oral disease, which has a high worldwide prevalence despite the availability of various prophylactic means, including the daily use of fluoride toothpastes, water fluoridation, dental sealants, oral health educational programs and various antiseptic mouthrinses. One important reason for this is uncontrolled increase in consumption of foods containing considerable sucrose concentration, especially among children. Sucrose is easily metabolized by oral bacteria (mostly streptococci) to acids and, subsequently, causing tooth decay or dental caries. In the oral ecosystem, streptococci principally reside on tooth surfaces forming biofilm. Important structural and binding materials of biofilm are glucan polymers synthesized by several isoforms of glucosyltransferase enzyme present in certain species of oral bacteria, including mutans group streptococci – *Streptococcus mutans* and *Streptococcus sobrinus*, which preferably colonize humans. Thus, there is a constant need to develop the methods and chemotherapeutics for improving oral health care and decreasing teeth decay through the suppression of cariogenic biofilm formation in the oral cavity. The aim of this paper was to review literature related to the pathogenesis of dental caries as well as currently existing and experimental pharmaceutical substances used for prevention of this process.

Key words: dental caries, biofilm, Streptococcus, glucosyltransferase, sucrose, glucan.

INTRODUCTION

Dental caries continues to be one of the most prevalent human diseases in spite of various available prophylactic means (1, 2). It has a multifactorial etiology, including endogenous as well as exogenous causal and modifying factors. Certainly, the virulence of oral bacteria and some disorders within the host immune system may be important factors (3-5). However, the major critical element is exogenous and relating to dietary intake of fermentable carbohydrates and in particular sucrose, that is frequently found in high concentrations in sweets, biscuits, snacks, sweet drinks, etc. (6). Although it is difficult to control human behavior, many caries-preventive measures have been designed, including the daily use of fluoride toothpastes and antiseptic mouthrinses, water fluoridation, dental sealants, oral health educational programs as well as regular visits to dentist office. However, the recent epidemiological studies show the trend to global increase in dental caries that clearly indicate a need for development of new and effective prophylactic approaches (1, 2). It has a great importance taking into consideration all heavy expenses for dental treatment, which in many countries are not covered (or covered only in part) by governmental health care programs.

Thus, this review paper provide recent scientific information about the bacteria related to the pathogenesis of dental caries, mechanism of cariogenic biofilm formation and pharmaceuticals for the caries prevention.

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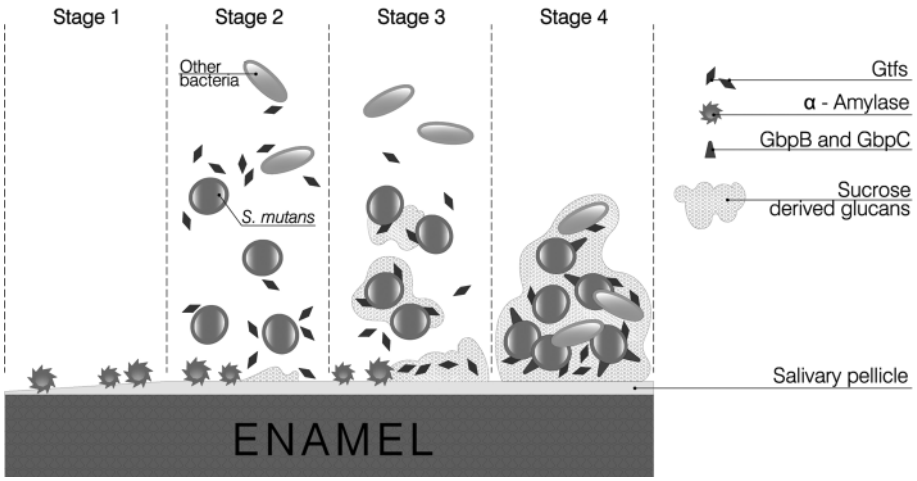

Fig. 1. Schematic representation of sucrose-dependent cariogenic biofilm formation on tooth enamel surface. This figure does not depict early colonizers of the enamel surface such as *S. oralis, S. sanguinis, S. gordonii, Actinomyces spp.* because their adherence is independent of sucrose. See other details in the text.

BACTERIA AND CARIOGENIC BIOFILM FORMATION

Bacteria involved in the pathogenesis of dental caries

Now it is well established that dental caries – a chronic infection directly related to certain species of commensal oral bacteria (3-5). It can be defined as a slowly progressive decay of tooth hard tissues (enamel, dentin) due to the dissolution of mineral components in effect of organic acids produced by the bacteria metabolizing sucrose and other food carbohydrates. Among the main etiologic pathogens involved in the pathogenesis of human dental caries are the mutans group streptococci (*Streptococcus mutans*, *S. sobrinus*), salivarius group streptococci (*S. salivarius*, *S. vestibularis*) and *S. parasanguinis* as well as lactobacilli (*L. gasseri*, *L. johnsonii*, *L. casei*, *L. paracasei*) and *Veillonella* species (*V. atypica*, *V. dispar*, *V. parvula*) (3-5, 7, 8). For the efficient colonization of surfaces, these bacteria primarily need to adhere to teeth and form biofilm (dental plaque). In this initial process, mutans streptococci (especially *S. mutans*) take the essential part by generating glucan polymers while other oral streptococci participate (9-11).

Glucans represent the polysaccharides composed of repeating glucose units, which are synthesized from sucrose by the enzymatic action of glucosyltransferases (Gtfs) and can be water-insoluble and water-soluble (9-12). They serve as a matrix for the biofilm with several functions: 1) promote bacterial adherence and further accumulation on teeth; 2) provide structural carcass to the biofilm; 3) increase acidogenicity of the biofilm matrix, as it is further described in details within the paper (10-13). Unlike others, *S. mutans* produces three types of the glucan polymers – water-insoluble glucan with alpha (α)-1,3 glucosidic linkages, partly water-soluble glucan containing a mixture of α-1,3 and α-1,6 glucosidic linkages as well as water-soluble glucan with α-1,6 glucosidic linkages, which are synthesized by GtfB, GtfC and GtfD enzymes, respectively (4, 9-11). There are three genes – *gtfB*, *gtfC* and *gtfD* that codes for GtfB, GtfC and GtfD enzymes, accordingly (4, 9-11). It is important to mention that, except *S. mutans*, the number of human oral streptococci have Gtfs and can produce glucans. *S. sobrinus* contains three Gtf (GtfU, GtfT, GtfS) enzymes for the generation of water-soluble glucans, and one Gtf (GtfI) enzyme for the production of water-insoluble glucan (9, 14). Contrarily, *S. oralis*, *S. sanguinis* and *S. gordonii* express a single Gtf that synthesizes glucans with various proportions of α-1,3 and α-1,6 linkages (15-17). In addition, *S. salivarius* has two Gtfs for the production of water-insoluble glucans as well as two Gtfs for the synthesis of water-soluble glucans (18). Finally, *S. parasanguinis* also possesses the Gtf1/2/3 enzyme complex but its function is related to the glycosylation of bacterial serine-rich glycoproteins involved in adhesion and biofilm development (19). Though *S. mutans* GtfB, GtfC activity and water-insoluble glucan are the most important for building the biofilm structure, the other isoforms of Gtf and glucan produced by various oral streptococci contribute to the adhesive biofilm formation in the synergistic manner (20). On the other hand, it should be highlighted that mutans streptococci (particularly *S. mutans*) producing Gtfs, glucans and biofilm are one of the primary and major etiologic factors implicated in the pathogenesis of dental caries. Taking this into consideration, it is critical to find and develop the efficacious caries preventive pharmaceutical agents inhibiting specifically the cariogenic biofilm formation through suppression of the streptococcal Gtfs and glucan synthesis.

Cariogenic Biofilm Formation

The dental biofilm or plaque is a community of oral bacteria infixed within polysaccharide matrix adherent to the tooth surface (5, 10). It contains water-insoluble glucan (10–20% of dry weight), fructan (1–2% of dry weight), bacterial and salivary proteins

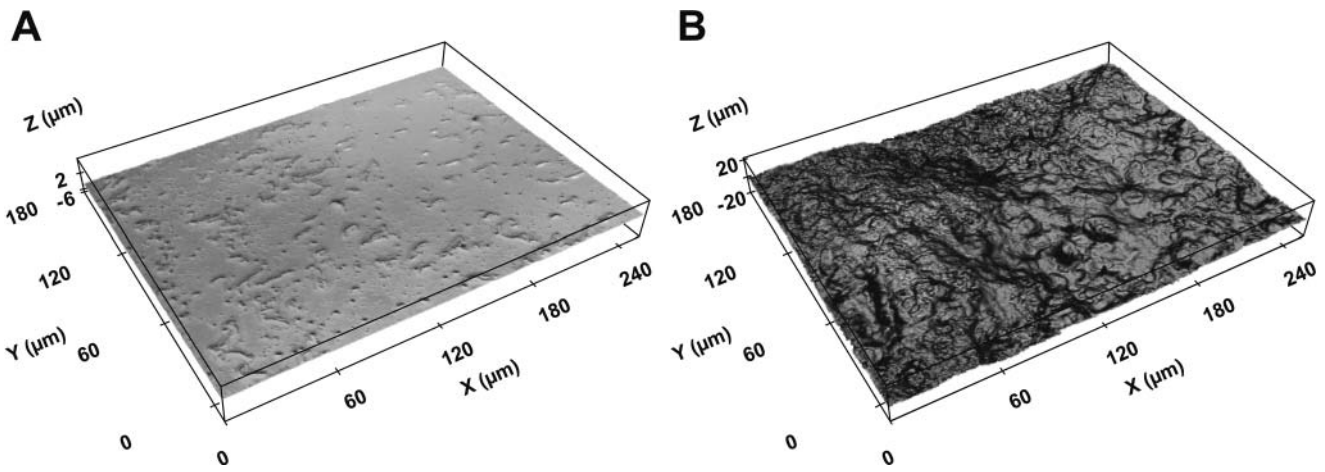

Fig. 2. Effect of sucrose on biofilm formation by mutans streptococci. A – optical profilometry of a glass slide surface in a mixed *S. mutans* and *S. sobrinus* culture after 24 h of incubation in Todd Hewitt broth without sucrose. B – optical profilometry of a glass slide surface with biofilm of a mixed *S. mutans* and *S. sobrinus* culture after 24 h of incubation in Todd Hewitt broth containing 1% sucrose. Photomicrographs are taken employing profilometer Sensofar PLu 2300 with 50× confocal objective at the Laser Research Center, Vilnius University, Lithuania.

(~40% of dry weight), variable quantities of lipid, calcium, phosphorus, magnesium, fluoride, and *in situ* conditions – water up to 80% (11). The extracellular polysaccharides in dental biofilm are mainly composed of water-insoluble glucans derived from the interaction of Gtfs with sucrose and starch hydrolysates (e.g., maltose). Importantly, the density of polysaccharide matrix increases following exposure of the bacterial biofilm to sucrose, and indeed a starch, which is also present in food, accelerates this process (21).

Formation of the biofilm on tooth surface is a multi-stage process, as described by Bowen and Koo (11), and illustrated in Fig. 1. In stage 1, at the beginning, salivary proteins are selectively adsorbed to enamel hydroxyapatite, including proline-rich proteins, alpha (α)-amylase, lysozyme, histatins, peroxidase, statherin and mucin 2. This initial layer of material is named as salivary pellicle, to which bacteria and their Gtfs attach. During stage 2, *S. mutans* secreted GtfC enzymes are attached and inserted into pellicle, subsequently synthesizing partly water-soluble glucans, thus promoting adhesion of the bacteria and GtfB proteins. Noteworthy, the GtfB enzymes produced by *S. mutans* can also adsorb on other bacterial surfaces, involving bacteria that do not express glucosyltransferases (e.g., *Actinomyces viscosus*, *Lactobacillus casei*). In stage 3, *S. mutans* GtfB and GtfC enzymes adhered to the surfaces readily and quickly utilize sucrose and generate water-insoluble and partly water-soluble glucans. The GtfD enzyme contributes to this process by synthesizing water-soluble glucans, which are employed as primers for GtfB, thereby increasing the entire production of extracellular polysaccharides. In parallel, if starch is available in the microenvironment, then it can be digested by α-amylase generating maltose, maltotriose, maltodextrin and other oligosaccharides that are included into the glucan polymer via acceptor reactions by GtfB. During stage 4, *S. mutans* glucan-binding proteins, i.e. GbpB and GbpC, as well as other bacteria bind to the glucan molecules resulting in stronger bacterial adhesion and consequently development of the microcolonies on surface of the tooth enamel. Furthermore, the glucosytransferases secreted by other streptococcal species and Gtf-adsorbed bacteria accompany *S. mutans* in glucan synthesis from sucrose contribute to the maturation of dental plaque.

Thus, it can be stated that indeed sucrose is the main triggering factor for development of the bacterial biofilm, as depicted by Marsh et al. (5) and clearly visualized in Fig. 2. When the biofilm is matured, then the presence of sucrose and/or starch further promotes plaque cariogenicity by constantly keeping pH at 5 or even lower (10, 11). Such persistent acidic environment within the biofilm results in demineralization of tooth enamel, that is, organic acids (e.g., lactic, acetic acids) produced during fermentation of sucrose penetrate into the enamel through the aqueous phase between hydroxyapatite crystals causing the dissolution of calcium along with phosphate (22, 23). Consequently, this long-term process leads to cavitation. Importantly, the acidic conditions within biofilm favors the growth of more acid-tolerant bacteria such as mutans streptococci and lactobacilli (10-12). Hence, the reasons delineated above determine why the biofilm formed on the tooth surface possesses cariogenicity.

In this context, it is significant to review current literature on pharmaceuticals that are used or can be applied for the inhibition of cariogenic biofilm formation.

PHARMACEUTICALS FOR THE PREVENTION OF DENTAL PLAQUE AND CARIES DEVELOPMENT

Chemotherapeutics for Dental Caries Prevention

Currently available chemotherapeutic agents have been proven to be effective in prevention of cariogenic biofilm formation in the oral cavity as well as dental caries prophylaxis, if applied according to directions (24, 25) (Table). Most of these agents exert an indirect effect on the biofilm development by inhibiting the growth of oral bacteria, and include various fluoride compounds (e.g., sodium fluoride, stannous fluoride), such chemical substances as

Table. Examples of chemical compounds used in clinical practice and experimental studies for the prevention of cariogenic biofilm and dental caries development *(continued on next page)*

Chemical compound	Target area of action	Principal mode of action	Effect on dental biofilm development	Clinical efficacy	References
Simple inorganic chemical compounds					
Sodium fluoride, stannous fluoride	Tooth hard tissues: enamel, dentin	Suppression of demineralization and stimulation of remineralization due to formation of fluorapatite	No proven effect on dental biofilm formation	Prevent dental caries (strong evidences)	24-28
	Multiple cytoplasmic and membrane enzymes of bacteria	Inactivation of enolase, F-ATPase leading to suppression of metabolism and acid tolerance			
Zinc compounds (e.g., zinc oxide)	S. mutans GtfB, GtfC, GtfD and S. sobrinus GtfI enzymes	Inactivation of Gtfs because of the binding to fructosyl site in catalytic domain leading to inhibition of glucan synthesis	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	11 (see references therein), 45
Copper compounds (e.g., copper amalgam)	S. mutans gtfB, gtfC genes and S. sobrinus GtfI enzyme	Downregulation of S. mutans gtfB, gtfC genes' expression and inactivation of S. sobrinus GtfI activity causing inhibition of glucan production	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	11 (see references therein), 59
Complex synthetic organic chemical compounds					
Chlorhexidine	Bacterial cell membrane	Disruption of cell membrane integrity causing bacteriolysis	Inhibits dental biofilm formation due to bacteriostatic and bactericidal effects	Provides cariostatic effect only in combination with fluoride compounds (moderately strong evidences)	24, 29, 30, 32
Triclosan	Multiple cytoplasmic and membrane enzymes of bacteria	Inactivation of enoyl-ACP reductase, pyruvate kinase, F-ATPase leading to suppression of fatty acids' synthesis, glycolysis and acid tolerance	Inhibits dental biofilm formation due to bacteriostatic and bactericidal effects	Provides cariostatic effect only in combination with fluoride compounds (moderately strong evidences)	24, 31, 33
Deoxynojirimycin, tris (hydroxymethyl) aminomethane, trichlorogalactosucrose, cetylpyridinium chloride, alexidine dihydrochloride	S. mutans GtfB, GtfC, GtfD and S. sobrinus GtfI enzymes	Inactivation of Gtfs because of the binding to glucosyl site in catalytic domain leading to inhibition of glucan synthesis	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	11 (see references therein), 45,
Methacryloxylethyl cetyl dimethyl ammonium chloride, quaternary ammonium dimethacrylate	S. mutans gtfB, gtfC genes	Downregulation of S. mutans gtfB, gtfC genes' expression	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	46, 47

chlorhexidine and triclosan incorporated in the composition of toothpastes, mouthrinses, tablets and varnishes (24). Fluoride is considered to be an efficient anticaries agent because of the several mechanisms of action: 1) suppression of the demineralization, that is, reduction of the solubility of tooth minerals – by substituting hydroxyl groups within calcium hydroxyapatite structure, and leading to the formation of more acid-resistant fluorapatite mineral; 2) stimulation of the remineralization – by constantly adsorbing it along with calcium and phosphate ions to the tooth surface from saliva, and resulting in the development of fluorapatite-like mineral; 3) inhibition of the bacterial metabolism (i.e. antibacterial

Table. Examples of chemical compounds used in clinical practice and experimental studies for the prevention of cariogenic biofilm and dental caries development *(continued from previous page)*

Chemical compound	Target area of action	Principal mode of action	Effect on dental biofilm development	Clinical efficacy	References
Phosphorothioate-modified antisense oligodeoxyribonucleotide	S. mutans gtfB mRNA	Selective inactivation of S. mutans gtfB mRNA function because of specific binding to its initial code region	Inhibits dental biofilm formation due to reduced production of water-insoluble glucans	No proven effect for prevention of dental caries	65
Complex natural organic chemical compounds					
Catechin-based polyphenols: epicatechin, epigallocatechin, epigallocatechin gallate	Gtf enzymes of mutans streptococci (S. mutans, S. sobrinus)	Inactivation of Gtfs because of conjugation with glucan binding domain, and their precipitation leading to inhibition of glucan synthesis	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	43 (see references therein)
Proanthocyanidins (i.e. epicatechin polymers)					
Gallotannins					
Benzophenones (e.g., 7-epiclusianone)	S. mutans GtfB, GtfC enzymes	Inactivation of S. mutans GtfB, GtfC because of the binding to catalytic domain leading to inhibition of glucan synthesis	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	43 (see references therein), 51
Flavonoids (e.g., apigenin)					
Anthraquinones: resveratrol, emodin, physcion	S. mutans F-ATPase enzyme, gtfB, gtfC genes and GtfB, GtfC enzymes	Inactivation of S. mutans F-ATPase, GtfB, GtfC activities and downregulation of gtfB, gtfC genes' expression leading to suppression of acid tolerance and glucan synthesis	Inhibits dental biofilm formation due to reduced production of glucans	No proven effect for prevention of dental caries	53-55
Terpenoids: thymol, 4-epi-pimaric acid, kaurenoic acid					
Vaccines					
Subunit vaccine	S. mutans cell-surface protein (PAc), glucan-binding proteins (Gbp), GtfB and S. sobrinus GtfI enzymes	Induction of salivary IgA antibody synthesis leading to inactivation of PAc, Gbp, GtfB and GtfI activities	Inhibits dental biofilm formation due to reduced bacterial adhesion and production of glucans	No proven effect for prevention of dental caries	60, 61, 63
Fusion DNA vaccine					

effect) – by binding and inactivating many of the enzymes involved in different metabolic pathways of bacteria (26). In addition, it has been recently found that fluoride also exerts a direct effect on *S. mutans* biofilm formation due to the attenuation of water-insoluble glucan production that is most likely related with the suppressed secretion of GtfB and GtfC through the bacterial cell membrane (27). However, this finding is controversial because the newest study carried out by the same researchers – Pandit et al. (28) did not show any effect of fluoride on the activity of *S. mutans* glucosyltransferase *in vitro*.

Contrarily, chlorhexidine (1,1'-hexamethylene bis (5-[4-chlorophenyl] biguanide)) and triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) possess mainly a broad-spectrum antimicrobial activity (24, 29-31). Depending on the concentration, chlorhexidine causes alterations of the bacterial cell membrane with the leakage of intracellular constituents (bacteriostatic effect) or induces irreversible precipitation of the cytoplasmic contents (bactericidal effect), whereas triclosan inhibits multiple cytoplasmic and membrane enzymes involved in the synthesis of fatty acids (enoyl-ACP reductase), glycolysis (pyruvate kinase) as well as acid tolerance (F-ATPase). Importantly, all these anticaries agents provide the best efficacy when they are applied in the combined fashion rather than separately (32, 33). On the other hand, although many of the fluoride, chlorhexidine and triclosan containing compounds are approved for the application in personal care and clinical practice, they can be toxic and/or cause side effects, especially if used for a long-time or inappropriately. Indeed, the excess exposure to fluoride leads to the development of dental and skeletal fluorosis (34). The long-term use of chlorhexidine results in teeth staining accompanied with unpleasant taste, and, in experimental conditions, it exhibits toxicity for osteoblast-like cells (29, 35). Finally, triclosan can affect endocrine system by exerting estrogenic and androgenic activity (36). However, most importantly, following the prolonged and persistent usage of the chemotherapeutics, oral bacteria can potentially acquire resistance to these agents either by means of an unstable phenotypic adaptation or a stable genetic alteration (37-42). Taken together, these facts definitely emphasize the need for continuous development of new anticariogenic substances with alternative modes of action.

Synthetic, Natural and Experimental Glucosyltransferase Inhibitors

In order to overcome the above-mentioned problems, there is made a substantial progress in the design of novel pharmaceuticals that provide a direct effect on the cariogenic biofilm formation, principally through the inhibition of *S. mutans* and *S. sobrinus* Gtfs' expression and/or activity (Table). Most of these Gtf inhibitors are still in experimental stages, and they can be classified into synthetic and natural compounds, as extensively reviewed by Bowen and Koo (11), and Jeon et al. (43). Streptococcal Gtfs structurally and functionally are complex enzymes possessing the N-terminal catalytic domain for conjugation of sucrose and its subsequent hydrolysis as well as the C-terminal glucan-binding domain for generation of glucan polymer (44, 45). The catalytic domain contains the glucosyl and fructosyl active sites which have been identified to be the suitable targets for synthetic inhibitors. In this respect, it has been demonstrated that such chemically synthesized compounds as deoxynojirimycin, tris(hydroxymethyl)aminomethane, trichlorogalactosucrose, cetylpyridinium chloride and alexidine dihydrochloride effectively inhibits activity of the Gtfs adsorbed to solid surface and/or in solution by likely interfering with the formation of glucose transition molecules within the glucosyl site (11). Moreover, some novel synthetic materials, like methacryloxylethyl cetyl dimethyl ammonium chloride and quaternary ammonium dimethacrylate, which can be incorporated in the adhesive system of dental composites, exhibit a significant antibiofilm effect *in vitro* via the selective suppression of *S. mutans gtfB* and *gtfC* genes' expression (46, 47). On the other hand, it is known that certain biodegradation products – methacrylic acid and triethylene glycol released from dental composites actually promote *S. mutans* biofilm formation through the same mechanism (48, 49).

As compared with synthetic inhibitors, there is a considerably greater number of natural substances derived mostly from plant extracts which exert the suppressive effects on streptococcal Gtfs and glucan production (43). These natural active compounds are identified as the catechin-based polyphenols, proanthocyanidins, gallotannins, benzophenones and flavonoids. It has been evidently shown that the catechin-based polyphenols – epicatechin, epigallocatechin, epigallocatechin gallate isolated from black, green and oolong teas, respectively, as well as proanthocyanidins (i.e. the epicatechin polymers found in cacao bean husks, cranberry fruits) and neem (*Azadirachta indica*) gallotannins effectively reduce the cariogenic biofilm formation by mutans streptococci *in vitro* and *in vivo* through a direct inhibition of the Gtf activities (43). Their mechanism of action is most likely related to the ability to conjugate with glucan-binding domain of

the Gtfs and precipitate the enzymes within solution, thereby resulting in the inactivation. Noteworthy, recent findings of Xu et al. (50) indicate that tea polyphenol – the epigallocatechin gallate can also suppress the development of *S. mutans* biofilm by simultaneously inhibiting the expression of *gtfB*, *gtfC* and *gtfD* genes. Similarly, the 7-epiclusianone (i.e. a benzophenone found in the fruits of *Rheedia brasiliensis*) and apigenin, which is a flavonoid derived from propolis, exhibit the potent anti-Gtf activities, however because of the binding with a catalytic domain of the Gtf enzymes (43, 51). Current researches have identified and revealed that the anthraquinones (i.e. resveratrol, emodin, physcion) found in the roots of Japanese knotweeds (*Polygonum cuspidatum*), terpenoids – thymol separated from ajowan seeds (*Trachyspermum ammi*), 4-*epi*-pimaric and kaurenoic acids isolated from spikenards (*Aralia cachemirica*, *A. continentalis*) as well as polyphenol-rich extracts of the Japanese wild grape (*Vitis coignetiae*) pomace, strawberry guava (*Psidium cattleianum*) leaf and rock cinquefoil (*Drymocallis rupestris*) aerial part possess significant inhibitory effects on the biofilm formation by mutans streptococci, principally due to reduction of the exopolysaccharide synthesis (52-58). In addition, such naturally and artificially occurring metal ions as zinc and copper have been found to suppress function of the Gtfs at different levels. Specifically, zinc ions inactivate the Gtf enzymes because of direct binding to fructosyl site in the catalytic domain, whereas copper ions downregulate the expression of *S. mutans gtfB* and *gtfC* genes at the transcriptional level (11, 59). Importantly, it should be noted that the antibiofilm and anticariogenic activities of certain natural compounds (e.g., benzophenones, anthraquinones) are substantially enhanced when they are combined with fluoride rather than apart (43, 51, 52). However, although all natural agents are very attractive for the application in dental caries prophylaxis and/or treatment because of their inartificial origin, they usually possess multiple effects, including bacteriostatic and bactericidal, which are undesirable in such complex and sensitive ecosystem of the oral bacteria (43).

Ultimately, in the context of the reviewed articles, there are rapidly evolving modern pharmaceutical technologies based on bioinformatics that enable researchers to design pharmacological agents precisely targeting only the Gtfs of mutans streptococci (60). In terms of this, the most promising biopharmaceuticals are considered to be the vaccines because they can provide a long-term protection against the cariogenic bacteria (61). However, despite of the fact that different dental caries vaccines were developed several decades ago, their protective effectiveness was insufficient in the practice (4). Indeed, it could be attributed to the poorly adjusted targets (including the Gtfs) of the old generation vaccines. The newest *in silico* and biotechnological methods have allowed to design and construct the DNA vaccines which are capable to inactivate simultaneously the Gtfs producing water-insoluble glucan of mutans streptococci (*S. mutans*, *S. sobrinus*), thereby reducing caries scores in animal models (62, 63). In addition, the novel antisense technology provides a possibility to inhibit selectively the expression of streptococcal glucosyltransferases at the transcriptional level (64). In this respect, Guo et al. (65) have demonstrated that the formation of cariogenic biofilm *in vitro* can be effectively suppressed with the phosphorothioate-modified antisense oligodeoxyribonucleotides specifically targeting and altering the mRNA transcribed from *S. mutans gtfB* gene. On the other hand, it should be taken into consideration that an efficacy of these state-of-the-art technologies still has to be proven in the clinical practice.

CONCLUSIONS

Several conclusions can be made in accordance with the reviewed literature. The first is that *S. mutans* appears to be a main etiologic agent involved in the pathogenesis of dental caries in humans because of the produced glucosyltransferases and glucans. Secondly, dietary sucrose is the major initiating factor for cariogenic biofilm formation on tooth surfaces. Finally, due to the reason that oral bacteria can potentially acquire resistance to currently used chemotherapeutics, there is a constant need for developing new pharmaceuticals with the selective anti-Gtf activities in order to prevent dental caries in humans.

REFERENCES

1. Marcenes W, Kassebaum NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, et al. Global burden of oral conditions in 1990-2010: a systematic analysis. *J Dent Res* 2013;92:592-7.
2. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. *Am J Dent* 2009;22:3-8.
3. Marsh PD. Are dental diseases examples of ecological

catastrophes? *Microbiology* 2003;149:279-94.
4. Quivey RG, Jr. Caries. In: Lamont RJ, Burne RA, Lantz MS, LeBlanc DJ. Oral microbiology and immunology. ASM Press; 2006. p. 233-52.
5. Marsh PD, Moter A, Devine DA. Dental plaque biofilms: communities, conflict and control. *Periodontol 2000* 2011;55:16-35.
6. Anderson CA, Curzon ME, Van Loveren C, Tatsi C, Duggal MS. Sucrose and dental caries: a review of the evidence. *Obes Rev* 2009;10(Suppl.1):41-54.
7. Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, et al. Bacterial 16S sequence analysis of severe caries in young permanent teeth. *J Clin Microbiol* 2010;48:4121-8.
8. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond Streptococcus mutans: dental caries onset linked to multiple species by 16S rRNA community analysis. *PLoS ONE* 2012;7:e47722.
9. Nishimura J, Saito T, Yoneyama H, Bai LL, Okumura K, Isogai E. Biofilm formation by Streptococcus mutans and related bacteria. *Adv Microbiol* 2012;2:208-15.
10. Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. *J Dent Res* 2013;92:1065-73.
11. Bowen WH, Koo H. Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. *Caries Res* 2011;45:69-86.
12. Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA. The role of sucrose in cariogenic dental biofilm formation - new insight. *J Dent Res* 2006;85:878-87.
13. Xiao J, Klein MI, Falsetta ML, Lu B, Delahunty CM, Yates III JR, et al. The exopolysaccharide matrix modulates the interaction between 3D architecture and virulence of a mixed-species oral biofilm. *PLoS Pathog* 2012;8:e1002623.
14. Hanada N, Fukushima K, Nomura Y, Senpuku H, Hayakawa M, Mukasa H, et al. Cloning and nucleotide sequence analysis of the Streptococcus sobrinus gtfU gene that produces a highly branched water-soluble glucan. *Biochim Biophys Acta* 2002;1570:75-9.
15. Fujiwara T, Hoshino T, Ooshima T, Sobue S, Hamada S. Purification, characterization, and molecular analysis of the gene encoding glucosyltransferase from Streptococcus oralis. *Infect I*mmun 2000;68:2475-83.
16. Kopec LK, Vacca-Smith AM, Wunder D, Ng-Evans L, Bowen WH. Properties of Streptococcus sanguinis glucans formed under various conditions. *Caries Res* 2001;35:67-74.
17. Vickerman MM, Sulavik MC, Nowak JD, Gardner NM, Jones GW, Clewell DB. Nucleotide sequence analysis of the Streptococcus gordonii glucosyltransferase gene, gtfG. *DNA Seq* 1997;7:83-95.
18. Simpson CL, Cheetham NWH, Giffard PM, Jacques NA. Four glucosyltransferases, GtfJ, GtfK, GtfL and GtfM, from Streptococcus salivarius ATCC 25975. *Microbiology* 1995;141:1451-60.
19. Zhou M, Zhu F, Dong S, Pritchard D, Wu H. A novel glucosyltransferase is required for glycosylation of a serine-rich adhesin and biofilm formation by Streptococcus parasanguinis. *J Biol Chem* 2010;285:12140-8.
20. Tamesada M, Kawabata S, Fujiwara T, Hamada S. Synergistic effects of streptococcal glucosyltransferases on adhesive biofilm formation. *J Dent Res* 2004;83:874-9.
21. Klein MI, DeBaz L, Agidi S, Lee H, Xie G, Lin AH, et al. Dynamics of Streptococcus mutans transcriptome in response to starch and sucrose during biofilm development. *PLoS ONE* 2010;5:e13478.
22. Featherstone JDB. Dental caries: a dynamic disease process. *Aust Dent J* 2008;53:286-91.
23. Cross SE, Kreth J, Wali RP, Sullivan R, Shi W, Gimzewski JK. Evaluation of bacteria-induced enamel demineralization using optical profilometry. *Dent Mater* 2009;25:1517-26.
24. Rodrigues JA, Lussi A, Seemann R, Neuhaus KW. Prevention of crown and root caries in adults. *Periodontol 2000* 2011;55:231-49.
25. Gluzman R, Katz RV, Frey BJ, McGowan R. Prevention of root caries: a literature review of primary and secondary preventive agents. *Spec Care Dentist* 2013;33:133-40.
26. Buzalaf MA, Pessan JP, Honório HM, ten Cate JM. Mechanisms of action of fluoride for caries control. *Monogr Oral Sci* 2011;22:97-114.
27. Pandit S, Kim JE, Jung KH, Chang KW, Jeon JG. Effect of sodium fluoride on the virulence factors and composition of Streptococcus mutans biofilms. *Arch Oral Biol* 2011;56:643-9.
28. Pandit S, Kim HJ, Song KY, Jeon JG. Relationship between fluoride concentration and activity against virulence factors and viability of a cariogenic biofilm: in vitro study. *Caries Res* 2013;47:539-47.
29. Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. *Minerva Stomatol* 2012;61:399-419.
30. Borges FM, de Melo MA, Lima JP, Zanin IC, Rodrigues LK. Antimicrobial effect of chlorhexidine digluconate in dentin: in vitro and in situ study. *J Conserv Dent* 2012;15:22-6.
31. Phan TN, Marquis RE. Triclosan inhibition of membrane enzymes and glycolysis of Streptococcus mutans in suspensions and biofilms. *Can J Microbiol* 2006;52:977-83.
32. De Amorim RG, Leal SC, Bezerra AC, de Amorim FP, de Toledo OA. Association of chlorhexidine and fluoride for plaque control and white spot lesion remineralization in primary dentition. *Int J Paediatr Dent* 2008;18:446-51.
33. Vered Y, Zini A, Mann J, De Vizio W, Stewart B, Zhang YP, 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 a root caries and dental crowns among adults. *J Clin Dent* 2009;20:62-5.
34. Jha SK, Mishra VK, Sharma DK, Damodaran T. Fluoride in the environment and its metabolism in humans. *Rev Environ Contam Toxicol* 2011;211:121-42.
35. Lessa FC, Aranha AM, Nogueira I, Giro EM, Hebling J, Costa CA. Toxicity of chlorhexidine on odontoblast-like cells. *J Appl Oral Sci* 2010;18:50-8.
36. Fang JL, Stingley RL, Beland FA, Harrouk W, Lumpkins DL, Howard P. Occurrence, efficacy, metabolism, and toxicity of triclosan. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2010;28:147-71.
37. Baker JL, Sudarsan N, Weinberg Z, Roth A, Stockbridge RB, Breaker RR. Widespread genetic switches and toxicity resistance proteins for fluoride. *Science* 2012;335:233-5.
38. Zhu L, Zhang Z, Liang J. Fatty-acid profiles and expression of the fabM gene in a fluoride-resistant strain of Streptococcus mutans. *Arch Oral Biol* 2012;57:10-4.
39. Ortega Morente E, Fernández-Fuentes MA, Grande Burgos MJ, Abriouel H, Pérez Pulido R, Gálvez A. Biocide tolerance in bacteria. *Int J Food Microbiol* 2013;162:13-25.
40. Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety. Chlorhexidine compounds in cosmetic products: risk assessment of antimicrobial and antibiotic resistance development in microorganisms. Norwegian Scientific Committee for Food Safety; 2010.
41. McBain AJ, Ledder RG, Sreenivasan P, Gilbert P. Selection for high-level resistance by chronic triclosan exposure is not universal. *J Antimicrob Chemother* 2004;53:772-7.
42. Kim TO, Im DW, Jung HY, Kwon SJ, Heo YS. Purification, crystallization and preliminary X-ray diffraction

analysis of enoyl-acyl carrier protein reductase (FabK) from Streptococcus mutans strain UA159. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2012;68(Pt 3):292-3.
43. Jeon JG, Rosalen PL, Falsetta ML, Koo H. Natural products in caries research: current (limited) knowledge, challenges and future perspective. *Caries Res* 2011;45:243-63.
44. Tsai YW, Chia JS, Shiau YY, Chou HC, Liaw YC, Lou KL. Three-dimensional modeling of the catalytic domain of Streptococcus mutans glucosyltransferase GtfB. *FEMS Microbiol Lett* 2000;188:75-9.
45. Devulapalle KS, Moosert G. Subsite specificity of the active site of glucosyltransferases from Streptococcus sobrinus. *J Biol Chem* 1994;269:11967-71.
46. Li F, Chai ZG, Sun MN, Wang F, Ma S, Zhang L, et al. Anti-biofilm effect of dental adhesive with cationic monomer. *J Dent Res* 2009;88:372-6.
47. Li F, Weird MD, Chen J, Xu HH. Comparison of quaternary ammonium-containing with nano-silver-containing adhesive in antibacterial properties and cytotoxicity. *Dent Mater* 2013;29:450-61.
48. Singh J, Khalichi P, Cvitkovitch DG, Santerre JP. Composite resin degradation products from BisGMA monomer modulate the expression of genes associated with biofilm formation and other virulence factors in Streptococcus mutans. *J Biomed Mater Res A* 2009;88:551-60.
49. Khalichi P, Sigh J, Cvitkovitch D, Santerre JP. The influence of triethylene glycol derived from dental composite resins on the regulation of Streptococcus mutans gene expression. *Biomaterials* 2009;30:452-9.
50. Xu X, Zhou XD, Wu CD. Tea catechin epigallocatechin gallate inhibits Streptococcus mutans biofilm formation by suppressing gtf genes. *Arch Oral Biol* 2012;57:678-83.
51. Murata RM, Branco-de-Almeida LS, Franco EM, Yatsuda R, dos Santos MH, de Alencar SM, et al. Inhibition of Streptococcus mutans biofilm accumulation and development of dental caries in vivo by 7-epiclusianone and fluoride. *Biofouling* 2010;26:865-72.
52. Pandit S, Kim HJ, Park SH, Jeon JG. Enhancement of fluoride activity against Streptococcus mutans biofilms by a substance separated from Polygonum cuspidatum. *Biofouling* 2012;28:279-87.
53. Khan R, Adil M, Danishuddin M, Verma PK, Khan AU. In vitro and in vivo inhibition of Streptococcus mutans biofilm by Trachyspermum ammi seeds: an approach to alternative medicine. *Phytomedicine* 2012;19:747-55.
54. Ali F, Sangwan PL, Koul S, Pandey A, Bani S, Abdullah ST, et al. 4-epi-Pimaric acid: a phytomolecule as a potent antibacterial and anti-biofilm agent for oral cavity pathogens. *Eur J Clin Microbiol Infect Dis* 2012;31:149-59.
55. Jeong SI, Kim BS, Keum KS, Lee KH, Kang SY, Park BI, et al. Kaurenoic acid from Aralia continentalis inhibits biofilm formation of Streptococcus mutans. *Evid Based Complement Alternat Med* 2013;2013:160592.
56. Yano A, Kikuchi S, Takahashi T, Kohama K, Yoshida Y. Inhibitory effects of the phenolic fraction from the pomace of Vitis coignetiae on biofilm formation by Streptococcus mutans. *Arch Oral Biol* 2012;57:711-9.
57. Brighenti FL, Gaetti-Jardim EJr, Danelon M, Evangelista GV, Delbem AC. Effect of Psidium cattleianum leaf extract on enamel demineralisation and dental biofilm composition in situ. *Arch Oral Bi*ol 2012;57:1034-40.
58. Tomczyk M, Pleszczyńska M, Wiater A, Granica S. In vitro anticariogenic effects of Drymocallis rupestris extracts and their quality evaluation by HPLC-DAD-MS3 analysis. *Molecules* 2013;18:9117-31.
59. Chen PM, Chen JY, Chia JS. Different regulation of Streptococcus mutans gtfBCD genes in response to copper ions. *Arch Microbiol* 2006;185:127-35.
60. Chen F, Wang D. Novel technologies for the prevention and treatment of dental caries: a patent survey. *Expert Opin Ther Pat* 2010;20:681-94.
61. KT S, KMK M, N B, Jimson S, R S. Dental caries vaccine - a possible option? *J Clin Diagn Res* 2013;7:1250-3.
62. Hoshino T, Kondo Y, Saito K, Terao Y, Okahashi N, Kawabata S, et al. Novel epitopic region of glucosyltransferase B from Streptococcus mutans. *Clin Vaccine Immunol* 2011;18:1552-61.
63. Niu Y, Sun J, Fan M, Xu QA, Guo J, Jia R, et al. Construction of a new fusion anti-caries DNA vaccine. *J Dent Res* 2009;88:455-60.
64. Bai H, Xue X, Hou Z, Zhou Y, Meng J, Luo X. Antisense antibiotics: a brief review of novel target discovery and delivery. *Curr Drug Discov Technol* 2010;7:76-85.
65. Guo QY, Xiao G, Li R, Guan SU, Zhu XL, Wu JZ. Treatment of Streptococcus mutans with antisense oligodeoxyribonucleotides to gtfB mRNA inhibits GtfB expression and function. *FEMS Microbiol Lett* 2006;264:8-14.

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