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.
REVIEWS

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**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 an 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 Form**

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...
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 glucosyltransferases 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.

(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
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)

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Target area of action</th>
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<th>Effect on dental biofilm development</th>
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<tr>
<td>Simple inorganic chemical compounds</td>
<td></td>
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<tr>
<td>Sodium fluoride, stannous fluoride</td>
<td>Tooth hard tissues: enamel, dentin</td>
<td>Suppression of demineralization and stimulation of remineralization due to formation of fluorapatite</td>
<td>No proven effect on dental biofilm formation</td>
<td>Prevent dental caries (strong evidences)</td>
<td>24-28</td>
</tr>
<tr>
<td>Zinc compounds (e.g., zinc oxide)</td>
<td>Multiple cytoplasmic and membrane enzymes of bacteria</td>
<td>Inactivation of enolase, F-ATPase leading to suppression of metabolism and acid tolerance</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>11 (see references therein), 45</td>
</tr>
<tr>
<td>Copper compounds (e.g., copper amalgam)</td>
<td>S. mutans GtfB, GtfC, GtfD and S. sobrinus Gtf enzymes</td>
<td>Inactivation of Gtfs because of the binding to fructosyl site in catalytic domain leading to inhibition of glucan synthesis</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>11 (see references therein), 59</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Bacterial cell membrane</td>
<td>Disruption of cell membrane integrity causing bacteriolysis</td>
<td>Inhibits dental biofilm formation due to bacteriostatic and bactericidal effects</td>
<td>Provides cariostatic effect only in combination with fluoride compounds (moderately strong evidences)</td>
<td>24, 29, 30, 32</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Multiple cytoplasmic and membrane enzymes of bacteria</td>
<td>Inactivation of enoyl-ACP reductase, pyruvate kinase, F-ATPase leading to suppression of fatty acids’ synthesis, glycolysis and acid tolerance</td>
<td>Inhibits dental biofilm formation due to bacteriostatic and bactericidal effects</td>
<td>Provides cariostatic effect only in combination with fluoride compounds (moderately strong evidences)</td>
<td>24, 31, 33</td>
</tr>
<tr>
<td>Deoxyxojirimycin, tris (hydroxymethyl) aminomethane, trichlorogalactosucrose, cetylpyridinium chloride, alexidine dihydrochloride</td>
<td>S. mutans GtfB, GtfC, GtfD and S. sobrinus Gtf enzymes</td>
<td>Inactivation of Gtfs because of the binding to glucosyl site in catalytic domain leading to inhibition of glucan synthesis</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>11 (see references therein), 45, 46, 47</td>
</tr>
</tbody>
</table>

| Complex synthetic organic chemical compounds | | | | | |
| Methacryloxyethyl 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 |
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<tr>
<td>Phosphorothioate-modified antisense oligodeoxynucleotide</td>
<td>S. mutans gtfB mRNA</td>
<td>Selective inactivation of S. mutans gtfB mRNA function because of specific binding to its initial code region</td>
<td>Inhibits dental biofilm formation due to reduced production of water-insoluble glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>65</td>
</tr>
<tr>
<td>Catechin-based polyphenols: epicatechin, epigallocatechin, epigallocatechin gallate</td>
<td>Gtf enzymes of mutans streptococci (S. mutans, S. sobrinus)</td>
<td>Inactivation of Gtfs because of conjugation with glucan binding domain, and their precipitation leading to inhibition of glucan synthesis</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>43 (see references therein)</td>
</tr>
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<td>Proanthocyanidins (i.e. epicatechin polymers)</td>
<td>S. mutans GtfB, GtfC enzymes</td>
<td>Inactivation of S. mutans GtfB, GtfC because of the binding to catalytic domain leading to inhibition of glucan synthesis</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>43 (see references therein), 51</td>
</tr>
<tr>
<td>Gallotannins</td>
<td>S. mutans F-ATPase enzyme, gtfB, gtfC genes and GtfB, GtfC enzymes</td>
<td>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</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>53-55</td>
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<td>Benzophenones (e.g., 7-epiclusianone)</td>
<td>S. mutans cell-surface protein (PAC), glucan-binding proteins (Gbp), GtfB and S. sobrinus GtfI enzymes</td>
<td>Induction of salivary IgA antibody synthesis leading to inactivation of PAC, Gbp, GtfB and GtfI activities</td>
<td>Inhibits dental biofilm formation due to reduced bacterial adhesion and production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>60, 61, 63</td>
</tr>
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<td>Flavonoids (e.g., apigenin)</td>
<td>S. mutans F-ATPase enzyme, gtfB, gtfC genes and GtfB, GtfC enzymes</td>
<td>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</td>
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<td>43 (see references therein), 51</td>
</tr>
<tr>
<td>Anthraquinones: resveratrol, emodin, physcion</td>
<td>S. mutans F-ATPase enzyme, gtfB, gtfC genes and GtfB, GtfC enzymes</td>
<td>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</td>
<td>Inhibits dental biofilm formation due to reduced production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>53-55</td>
</tr>
<tr>
<td>Terpenoids: thymol, 4-epi-pimaric acid, kaurenoic acid</td>
<td>S. mutans cell-surface protein (PAC), glucan-binding proteins (Gbp), GtfB and S. sobrinus GtfI enzymes</td>
<td>Induction of salivary IgA antibody synthesis leading to inactivation of PAC, Gbp, GtfB and GtfI activities</td>
<td>Inhibits dental biofilm formation due to reduced bacterial adhesion and production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
<td>60, 61, 63</td>
</tr>
<tr>
<td>Vaccines</td>
<td>S. mutans cell-surface protein (PAC), glucan-binding proteins (Gbp), GtfB and S. sobrinus GtfI enzymes</td>
<td>Induction of salivary IgA antibody synthesis leading to inactivation of PAC, Gbp, GtfB and GtfI activities</td>
<td>Inhibits dental biofilm formation due to reduced bacterial adhesion and production of glucans</td>
<td>No proven effect for prevention of dental caries</td>
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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...
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 anticiaries 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 anticiarogenic substances with alternative modes of action.

**Synthetic, Natural and Experimental Gluco-syltransferase 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 deoxyojoirimycin, 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 methacyrloxyethyl 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 epicathechin polymers found in cacao bean husks, cranberry fruits) and neem (Azadirachta indica) gallotannins effectively reduce the cariogenic biofilm formation by mutants 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 mutants 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 antibiotic 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 pharma-ceutical 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 mutants 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.

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