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REVIEW ARTICLE

Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity

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Glycosyltransferase (Gtf) is one of the crucial virulence factors of *Streptococcus mutans*, a major etiological pathogen of dental caries. All the available evidence indicates that extracellular polysaccharide, particularly glucans produced by *S. mutans* Gtfs, contribute to the cariogenicity of dental biofilms. Therefore, inhibition of Gtf activity and the consequential polysaccharide synthesis may impair the virulence of cariogenic biofilms, which could be an alternative strategy to prevent the biofilm-related disease. Up to now, many Gtf inhibitors have been recognized in natural products, which remain the major and largely unexplored source of Gtf inhibitors. These include catechin-based polyphenols, flavonoids, proanthocyanidin oligomers, polymeric polyphenols, and some other plant-derived compounds. Metal ions, oxidizing agents, and some other synthetic compounds represent another source of Gtf inhibitors, with some novel molecules either discovered by structure-based virtual screening or synthesized based on key structures of known inhibitors as templates. Antibodies that inhibit one or more Gtfs have also been developed as topical agents. Although many agents have been shown to possess potent inhibitory activity against glucan synthesis by Gtfs, bacterial cell adherence, and caries development in animal models, much research remains to be performed to find out their mechanism of action, biological safety, cariostatic efficacies, and overall influence on the entire oral community. As a strategy to inhibit the virulence of cariogenic microbes rather than eradicate them from the microbial community, Gtf inhibition represents an approach of great potential to prevent dental caries.

Keywords: *inhibitor*; *glycosyltransferase*; S. mutans; *dental caries*

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Dental biofilm, often described as a group of microorganisms embedded in an extracellular polysaccharide (EPS) matrix attached to the tooth surface, is responsible for multiple diseases occurring in the oral cavity (e.g. dental caries). Great attention is paid to the bacterially derived polysaccharide matrix in the pathogenesis of these biofilm-dependent diseases (1). The polysaccharide matrix can extensively modulate the virulence of biofilm by enhancing initial adherence of microorganisms and bacterial coherence, affecting transport of nutrients, acting as reserve supply of carbohydrate, and protecting the microbial community from inimical stimulus (2–6).

It is also recognized that a group of exoenzymes named glucosyltransferases (Gtfs) play critical roles in the synthesis of EPS, providing sites on dental surfaces for microbial colonization, adherent glucan for bacterial coherence, and water-insoluble matrix for biofilm formation. As a major etiological pathogen of dental caries, *Streptococcus mutans* is also regarded as the key contributor to EPS (4, 7). *S. mutans* expresses at least three genetically separate Gtfs: GtfB, which synthesizes primarily insoluble glucans rich in α-1,3 glycosidic linkages; GtfC, which produces a mixture of insoluble and soluble glucans (with α-1,6-linkages); and GtfD, which synthesizes predominantly soluble glucans. Each plays a distinct but overlapping role (8–10). GtfC has the greatest affinity for saliva-coated hydroxyapatite (sHA) with enhanced activity when it is adsorbed to sHA, thus enhancing the adherence of bacterial cells to dental surfaces, whereas GtfB binds with greater avidity to many oral bacteria including those that do not express Gtfs, thereby

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converting them into glucan formers and promoting cell accumulation in the biofilm (11). GtfD, however, synthesizes mainly water-soluble glucans, which serve as primers for GtfB (12). The formation and structure of glucans synthesized by Gtfs, particularly by GtfB, is also modulated by starch hydrolysates, which include a range of degradation products of starch digested by salivary α-amylase (13). The hydrolysates serve as acceptors for polymer formation and substrate for the addition of branches even without supply of sucrose (14–16).

It is noteworthy that a similar basic structure is found in all Gtfs encoded by *S. mutans* (11, 17, 18), particularly the highly homologous GtfB and GtfC, which shares approximately 75% amino acid sequence identity (19). The sequence of glucosyltransferase gene (*gtfI*) from cariogenic *Streptococcus sobrinus* also showed a high degree of homology with *gtfB* of *S. mutans* (17). Gtfs are large enzymes with an average molecular mass of approximately 160 kDa. All Gtfs are composed of three functional regions: the N-terminal variable junction region, the C-terminal glucan-binding region, and the highly conserved catalytic region, which is essential for glucan synthesis of Gtfs (20, 21). Gtf has at least two subsites, glucosyl and fructosyl, which could be potential targets for inhibitory agents (22). Recently, the crystallization and X-ray analysis for GtfC from *S. mutans* was reported, and the detailed structural information on GtfC catalytic domain was revealed (23). It is also noteworthy that GtfC, particularly its C terminus, has many hydrophobic domains (19), which can explain its remarkable resistance to inhibitory agents as these domains may block access by hydrophilic inhibitors to the active site. These researches provided instrumental data, opportunities, and also challenges for the development of novel chemotherapeutic agents targeting Gtfs to control biofilm-related diseases.

Following recognition of the etiologic importance and biology of Gtfs (7), inhibition of Gtf activity (particularly GtfC and GtfB) and polysaccharide synthesis as an approach to prevent biofilm-related diseases has started to draw attention. This approach has been advocated by Bowen and Koo (7) and many others. These enzymes are attractive drug targets for the development of therapeutics for dental caries, as such a selective approach on a proven virulence may confer selectivity against the pathogen with minimal effect on other commensal microbes. However, it is notable that an agent that inhibits Gtf in solution may not necessarily work when the enzyme is adsorbed to a surface (24) and agents effective *in vitro* may not have the capacity to modulate the pathogenesis of dental caries *in vivo*. The polyphenolic compounds derived from natural products have been studied extensively for years and were found to display potent antimicrobial activity by mechanisms, including the inhibition of Gtfs. Another strategy to inhibit polysaccharide synthesis is to screen or even design novel small molecules that bind specifically to the enzymatic site of Gtf and inhibit its catalytic activity. The aim of this current review is to provide an up-to-date account of researches into molecules (Table 1) that target bacterial polysaccharide synthesis by inhibition of Gtf activity.

Possible biological effects of administration of Gtf inhibitors

As a proven virulence factor in the pathogenesis of dental caries (4, 7), Gtfs and their catalysates play a significant role in the development of dental plaque. Therefore, a reduction in initial formation and accumulation of biofilm is the most possible effect resulting from impaired polysaccharide synthesis and poor sucrose-dependent adherence due to inhibited Gtf activity. Additionally, the loss of Gtf activity is likely to make the plaque more susceptible to mechanical clearance, which is also an essential mechanism for the cariostatic effect of Gtf inhibitors *in vivo*. Another overlooked advantage of Gtf-targeting inhibitors is their potential to possess anti-biofilm activity without being bactericidal, thus providing alternative methods to prevent biofilm-related diseases with minimal effect on the ecological balance in the microbial community.

Gtf inhibitors derived from natural products

Natural products offer a diversity of structurally distinctive molecules with a wide range of biochemical specificities, which make them attractive and a major objective for drug discovery (25). Among them, the polyphenols have been studied extensively in recent years, displaying anti-biofilm capacity by multiple mechanisms including direct inhibition of Gtf activity.

Catechin-based polyphenols

Various kinds of catechin-based polyphenols extracted from the leaves of *Camellia sinensis* (raw material of black, green, and oolong teas) have been shown to disrupt the enzymatic activity of Gtfs. Extracts of green tea, oolong tea and black tea were reported to reduce the development of caries on tooth surfaces in rats infected with *S. mutans* or *S. sobrinus* (26–28). Evidence *in vitro* indicated that the cariostatic effects of these compounds were related to their inhibitory effects of Gtfs rather than direct effects on the bacterial growth, with inhibitory activities of theaflavin, (-)-epigallocatechin gallate, and (-)-epicatechin gallate particularly noticeable (29–31). It was found that theaflavin derived from black tea extract produced 50% inhibition of purified GtfB in solution at the concentration of 8 μg/mL (10 μM) (30). (-)-Epigallocatechin gallate and (-)-epicatechin gallate were reported to inhibit 23 and 28% of GtfB activity (in solution) at the concentration of 250 μg/mL (546 and 566 μM, respectively) (30). Other possible mechanisms by

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Table 1. Molecules that inhibit glycosyltransferase activity

Category	Source of natural product	Representative substances	Inhibited Gtfs reported	Evidence of cariostatic efficacy *in vivo*	Reference
Inhibitors derived from natural products					
Catechin-based polyphenols	*Camellia sinensis* (black, green and oolong tea)	Theaflavin	GtfB (solution)	Yes (as crude tea extract)	
		(-)-Epigallocatechin gallate	GtfB (solution)	Yes (as crude tea extract)	(26–31)
		(-)-Epicatechin gallate	GtfB (solution)	Yes (as crude tea extract)	
Flavonoids	*Apis mellifera propolis* (bee hive)	Apigenin	GtfB, GtfC, and GtfD (solution/surface)	Yes	(36, 37)
		Kaempferol	GtfB, GtfC, and GtfD (solution/surface)	No	(36)
	Vaccinium macrocarpon (cranberry)	Myricetin	GtfB (surface)	No	(42)
Proanthocyanidin oligomers	Cranberry	A-type proanthocyanidin oligomers	GtfB (surface)	Yes	(42, 43)
	Apple	Condensed tannins	Crude Gtfs (solution)	No	(44)
Polymeric polyphenols	Oolong tea	OTF6	GtfB and GtfD (solution)	Yes (as crude tea extract)	(26, 48)
	Cocoa bean hust	Polymeric epicatechins	GtfB, GtfC, and GtfD (solution)	No	(49, 50)
Others	*Rheedia gardneriana*	7-Epiclusianone	GtfB and GtfC (solution)	No	(51)
	Azadirachta indica (Neem)	Gallotannin	Crude Gtfs (solution)	No	(52)
	Melaphis chinensis (Chinese Nutgall)	Gallotannin	Crude Gtfs (solution)	No	(53)
Synthesized inhibitors					
		Quaternary ammonium	Crude Gtfs (solution)	No	(54)
		Aliphatic amines	Crude Gtfs (solution)	No	(54)
		1-Deoxynojirimycin	GtfB, GtfC, and GtfD (solution/surface)	No	(24, 57)
		Tris(hydroxymethyl) aminomethane	Crude Gtfs (solution/surface)	No	(56)
		Trichloro-galactosucrose	GtfB, GtfC, and GtfD (solution/surface)	No	(24)
		Acarbose	GtfB, GtfD (solution)	No	(57)
		Maltose	GtfB (solution)	No	(57)
		Nojirimycin	GtfB (solution)	No	(57)
		Quinoxaline derivative	GtfC and GtfD (solution)	Yes	(58)
Metal ions and oxidizing agents		Zn^{2+}	GtfB, GtfC, and GtfD (solution)	No	(24)
		Cu^{2+}	GtfB, GtfC, and GtfD (solution/surface)	No	(24)
		Fe^{2+}	GtfB, GtfC, and GtfD (solution/surface)	No	(24)

Table 1 (Continued)

Category	Source of natural product	Representative substances	Inhibited Gtfs reported	Evidence of cariostatic efficacy *in vivo*	Reference
		Fe^{3+}	GtfB, GtfC, and GtfD (solution/surface)	No	(24)
		Rose bengal	GtfB, GtfC, and GtfD (solution/surface)	No	(24)
		Hypochlorite	GtfB, GtfC, and GtfD (solution/surface)	No	(24)
Antibodies		Antibody to GtfB	GtfB and GtfC (solution/surface)	Yes	(66, 68, 69)
		Antibody to GtfC	GtfB and GtfC (solution/surface)	No	(66)
		Antibody to GtfD	GtfD (solution), GtfC (surface)	No	(66)

which these catechin-based polyphenols are cariostatic include 1) direct antimicrobial bioactivity against *S. mutans*, 2) inhibitory effects on bacterial adherence and plaque accumulation, and 3) inhibition on acid production (32–35).

Flavonoids

It has recently been shown that specific flavonols (e.g. kaempferol and myricetin) and flavones (e.g. apigenin) in *Apis mellifera propolis* (bee hive) displayed inhibitory effects against Gtfs derived from *S. mutans* (36). Among them, apigenin (4′,5,7-trihydroxyflavone) was identified as the most effective non-competitive inhibitor of Gtfs, especially GtfB and GtfC, both in solution (90.5–95% inhibition) and on the surface of sHA (33–60.5% inhibition) at 500 μM (135 μg/mL) (36). Its cariostatic activity was also validated *in vivo* (37). Furthermore, it was speculated that the specific position of hydroxyl groups and the double bond between C2 and C3 may provide sites for nucleophilic addition by side chains of amino acid in Gtfs, which could diminish their activities (37). Similar inhibitory effects were observed for kaempferol on Gtfs, both in solution (86.7–90.4% inhibition) and on the surface of sHA (28.5–40.2% inhibition) at 500 μM (143 μg/mL) (37). Other biological activities possibly responsible for the cariostatic efficacy of flavonoids include 1) antimicrobial activity against planktonic cells and biofilms of *S. mutans*, 2) negative effects on acidogenic/aciduric properties, and 3) down-regulating *gtf* gene expression (36–41).

Proanthocyanidin oligomers

Aqueous extracts of cranberry have been shown to inhibit the synthesis of insoluble glucans by surface-adsorbed GtfB and GtfC, which was attributed mainly to the presence of flavonols and proanthocyanidin oligomers (42). According to Koo et al. (43), specific A-type proanthocyanidin oligomers (dimers to dodecamers) at the concentration of 0.1 mg/mL effectively diminished the synthesis of insoluble glucans by surface-adsorbed GtfB (40–70% inhibition) and the incidence/severity of smooth-surface caries in rats was markedly reduced by topical treatment of proanthocyanidins twice a day. Furthermore, the presence of A-type double interflavan linkage and a degree of polymerization (ranging from 4 to 12) was found to be optimal for the inhibition of surface-adsorbed enzyme (42, 43). Fruit-derived proanthocyanidin oligomers have also been recognized for their inhibitory activity against Gtfs of mutans streptococci. A study by Yanagida et al. has shown that apple polyphenols inhibit the activity of crude Gtfs in solution purified from *S. mutans* with no significant effect on the growth of the cariogenic bacteria (44). The most potent Gtf inhibitors in apple polyphenols were apple condensed tannins, a mixture of procyanidins,

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the 50% inhibitory doses (ID_{50}) of which against the crude Gtfs (in solution) of *S. sobrinus* and that of *S. mutans* were reported to be 1.5 and 5 μg/mL, respectively. Other possible mechanisms by which proanthocyanidin oligomers are caries preventing include: 1) antimicrobial activity against biofilms of *S. mutans*, 2) disruption of acidogenic/aciduric properties, and 3) enhanced detachment of biofilms of *S. mutans* (42, 43, 45–47).

Polymeric polyphenols

Matsumoto et al. demonstrated that the oolong tea fraction rich in high-molecular-weight polymeric polyphenols inhibited glucan synthesis by recombinant *S. mutans* GtfB and GtfD in solution non-competitively, with ID_{50} against GtfB and GtfD to be 60 and 100 μg/mL, respectively (48). They also reported that the mechanism of Gtf inhibition by these polymeric polyphenols was related to their activities targeting the C-terminal glucan-binding domain of GtfB and GtfD. Other researches showed that high-molecular-weight polymeric proanthocyanidins, consisting of epicatechin units with C-4β and C-8 intermolecular bonds, were the major compounds with inhibitory bioactivities against Gtfs in cocoa bean husk extracts (49). The crude ethanol extract inhibited the glucan synthesis of recombinant GtfB, C, and D in solution by 57%, 24%, and 26%, respectively, at the concentration of 1 mg/mL and the most active fraction, which contained 58.9% polyphenolic compounds, showed 68% inhibition at the concentration of 50 μg/mL(ID_{50} = 20 μg/mL). It was also reported that increasing polymerization of monomeric polyphenol was likely to improve their inhibitory effectiveness as a result of enhanced non-specific binding and precipitation of the enzymes (50).

Others

7-Epiclusianone, a prenylated benzophenone isolated from the plant *Rheedia gardneriana,* was reported to be a potent inhibitor of purified GtfB and GtfC in solution, with 91.7% inhibition of GtfB and 84.1% inhibition of GtfC at the concentration of 100 μg/mL (51). It was also revealed that the inhibition of GtfB was non-competitive, whereas GtfC was inhibited uncompetitively (51). Furthermore, the inhibitory effect of gallotannin in the aqueous extract derived from the sticks of neem (*Azadirachta indica*) upon insoluble glucan synthesis by mixed Gtfs in solution was shown by Wolinsky et al. (71% inhibition at 250 μg/mL) (52), while the same active constituent was also found in the ethanolic extracts from the Chinese Nutgall (*Melaphis chinensis*), exhibiting strong inhibition of crude Gtfs in solution of *S. sobrinus* origin with more than 91% inhibition of water-insoluble glucan synthesis from sucrose at the concentration of 7.8 μg/mL (53).

Synthesized Gtf inhibitors

Results from early work showed that wide-ranging synthesized compounds, such as quaternary ammonium compounds and aliphatic amines, were effective to varying degrees in inhibiting undetermined mixtures of Gtfs in solution (54). Meanwhile, 1-deoxynojirimycin, tris (hydroxymethyl) aminomethane, and trichloro-galactosucrose were shown to possess similar inhibitory activities against the Gtfs regardless of whether the enzymes were free in solution or adsorbed probably by binding to the aspartic acids in the active site and by blocking the formation of glucose transition molecules (24, 55–57). Among them, 1-deoxynojirimycin was more effective against either GtfB or GtfC than it was against GtfD and possessed similar inhibition profiles for Gtfs both in solution and those adsorbed to sHA (24). At 10 mM (163 μg/mL), the inhibitory effects on activities of solution-phase GtfB, C, and D were 33%, 44%, and 13%; and the numbers for surface-adsorbed GtfB, C, and D were 50%, 54%, and 11%, respectively (24). According to Newbrun et al. (57), acarbose effectively inhibited the synthesis of polysaccharide by *S. mutans* Gtf (at 20 mM, synthesis of water-insoluble glucan was completely blocked) and was more potent than other inhibitors such as maltose, nojirimycin, or amino sugars; and the mechanism of action of all these agents is consistent with competitive inhibition.

Recently, we screened a library of approximately 150,000 small drug-like compounds *in silico* against the structure of substrate-binding site of *S. mutans* GtfC and identified a quinoxaline derivative as a Gtf inhibitor (58). We found that at the concentration of 10 μg/mL (23.3 μM), the quinoxaline derivative was capable of inhibiting water-insoluble glucan synthesis (60% reduction) and biofilm formation (79% reduction) *in vitro*. Zymogram assays revealed that solution-phase GtfC and GtfD were antagonized by the compound. It was noticeable that it also significantly reduced the incidence and severity of caries in a rat model with a concomitant reduction of the percentage of *S. mutans* in the animals' dental plaque. This is the first report of Gtf inhibitor identified through structure-based virtual screening, and the structure identified could serve as a chemical entry point for further antibiotic development.

Another work was done more recently by Mesleh et al. (59), whose research had focused on the discovery of compounds with peptidoglycan glycosyltransferase inhibitory activity. Unlike the *S. mutans* Gtfs, this peptidoglycan glycosyltransferase enzyme transfers the disaccharide peptide from lipid II onto the growing glycan chain in the biosynthesis of bacterial cell wall peptidoglycan, thereby inhibiting the transglycosylation process results in the inhibition of bacterial growth in many Gram-positive organisms. They identified the minimal pharmacophore of a natural peptidoglycan glycosyltransferase inhibitor,

moenomycin, as the key feature required for inhibitory activity before using this structure as a template to design novel enzyme inhibitors. The ID_{50} of their most potent inhibitor against *Staphylococcus aureus* glycosyltransferase was as low as 7.3 μM.

Metal ions and oxidizing agents as Gtf inhibitors

Metal ions and some oxidizing agents have also been shown by some early studies to exhibit inhibitory activities against Gtfs. According to Wunder and Bowen (24), activities of isolated GtfB, C, and D of the solution phase were inhibited significantly by the metal ions Zn^{2+}, Cu^{2+}, Fe^{2+}, and Fe^{3+} (approximately 40–80% inhibition at 5 mM) and by rose bengal at 1 mM and hypochlorite at 10 mM (approximately 80–90% inhibition), whereas surface-adsorbed Gtfs displayed stronger resistance to the same metal cations and oxidizing compounds that inhibited them in solution. It is also clear that metal ions exert their inhibitory effects by taking advantage of a Fenton reaction in which they combine with peroxide to produce hydroxyl radical ions that are capable to inactivate Gtf enzymes (60).

Antibody

Streptococcus mutans Gtfs and their fragments were shown to be immunogenic (61), and antibodies that inhibit one or more Gtfs have been developed as topical agents.

Numerous antibody preparations were reported to inhibit sucrose-dependent aggregation, cell adhesion (62–64), and glucan synthesis by solution-phase GtfB, C, and D *in vitro* (65). Wunder and Bowen (66) used purified *S. mutans* GtfB, GtfC, or GtfD both in soluble form and surface-adsorbed to immunize rabbits and tested the antibodies for their inhibitory effects on Gtf activity. They found that antibodies to GtfB or GtfC inhibited the activity of solution-phase GtfB and GtfC by over 90% but had lesser effects against the same enzymes adsorbed to sHA (70–80%) and no effect on GtfD. Antibodies to GtfD had strong inhibitory activity against GtfD in solution (80–90%) and modest effects on GtfC bound to sHA, but no inhibition was observed for surface-adsorbed GtfD, solution-phase GtfC, and GtfB of both phases. Their data showed that antibodies effective against Gtf in one phase may react differently against the same enzyme in the other phase. It was also reported by Kopec et al. (67) that the structure of glucans synthesized by GtfB and GtfC in the presence of a polyclonal antiserum to mixed Gtfs differed from that of controls in glycosidic linkages, susceptibilities to mutanase/dextranase, and also hydrolysates from the enzymatic digestion.

Antibodies to various preparations of Gtf have been raised and applied topically to determine their effects on caries development *in vivo*. It was revealed that topical application of antibody to GtfB was effective in reducing the incidence of caries in rats (68, 69). It was also noted that passive immunization with bovine milk containing antibodies to hybrid GtfB and cell surface protein antigen (PAc) suppressed caries development in a rat model (70).

Conclusion

Since the cariogenic species are essential components in the microecological balance of oral flora, it would be not just unproductive but often counterproductive to eradicate them from the entire microbial population by antibiotic treatment. Therefore, it may be more rewarding to seek new strategies for inhibiting their key virulence without wiping them out. It is also well established that EPS are essential for the development of cariogenic biofilms and that mutans streptococci are the primary producers of the EPS-rich matrix through Gtfs, as reviewed by Koo et al. (1). Those exoenzymes synthesized by *S. mutans* are capable of converting many other species into cooperative EPS producers by adsorbing to their surfaces (11). Consequently, the elevated amounts of EPS will promote the accumulation of a number of different oral microorganisms on tooth surfaces and increase the stability of the biofilm, enhancing its virulence (71). Therefore, targeting the Gtf enzymes synthesized by *S. mutans* could be significant for therapeutic interventions to prevent dental caries, even though it is increasingly evident that caries is the result of the bacterial consortium acting together in the dental biofilm rather than the infection of a specific pathogen (72).

Other oral streptococci, such as *S. sanguinis*, *S. oralis*, and *S. gordonii*, also express Gtf enzyme and produce water-soluble glucans, which may contribute to the overall polysaccharide synthesis in the extracellular matrix and enhance adhesion of organisms producing water-insoluble glucans (73, 74). As our preliminary study showed, the inhibitor of *S. mutans* Gtfs resulted in poor biofilm formation for both *S. mutans* and *S. sanguinis,* but did not affect the biofilm formed by *S. gordonii* (58). In addition, the inhibitory profile on the formation of three-species biofilm was similar to those of the monospecies biofilms formed by *S. mutans* or *S. sanguinis* (58). However, evidence is still lacking that how the molecules targeting *S. mutans* Gtfs will affect the entire biofilm community, including the Gtf activities of other oral streptococci, and the possible effects on a health-associated microbiota. Therefore, much research remains to be performed from the point of microecology to represent a complete picture of the overall influence of this strategy on oral community.

Currently, a wide range of Gtf inhibitors have been discovered that can be broadly classed as either synthetic inhibitors or inhibitors from natural sources. With the possibility of screening large quantities of drug-like compounds against the active sites of Gtfs and designing new inhibitors using the key structures of known sites (both

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synthesized and natural) as templates, it is very likely that more effective molecules targeting Gtfs will continue to be discovered, and some of them may eventually be ready for therapeutic use. Although many of the available inhibitors are highly effective *in vitro*, it is still unclear whether they are suitable for *in vivo* use, and their cariostatic efficacies and mechanisms remain largely unknown. Therefore, further research is required to reveal the mechanism of ligand–enzyme interaction at the molecular level and assess treatments in animal experiments or even clinical trials before any conclusions can be made. In the meantime, comprehensive toxicological tests and safety studies should be performed both topically and systemically, even if the molecule is derived from natural products (traditional medicine, plant-derived foods, and so on), which are generally not expected to be toxic.

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References

1. Koo H, Xiao J, Klein MI. Extracellular polysaccharides matrix – an often forgotten virulence factor in oral biofilm research. Int J Oral Sci 2009; 1: 229–34.
2. Tatevossian A. Facts and artefacts in research on human dental plaque fluid. J Dent Res 1990; 69: 1309–15.
3. Wilson RF, Ashley FP. Relationships between the biochemical composition of both free smooth surface and approximal plaque and salivary composition and a 24-hour retrospective dietary history of sugar intake in adolescents. Caries Res 1990; 24: 203–10.
4. 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.
5. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010; 8: 623–33.
6. Koo H, Xiao J, Klein MI, Jeon JG. Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. J Bacteriol 2010; 192: 3024–32.
7. Bowen WH, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res 2011; 45: 69–86.
8. Aoki H, Shiroza T, Hayakawa M, Sato S, Kuramitsu HK. Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. Infect Immun 1986; 53: 587–94.
9. Hanada N, Kuramitsu HK. Isolation and characterization of the *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucans. Infect Immun 1988; 56: 1999–2005.
10. Hanada N, Kuramitsu HK. Isolation and characterization of the *Streptococcus mutans gtfD* gene, coding for primer-dependent soluble glucan synthesis. Infect Immun 1989; 57: 2079–85.
11. Vacca-Smith AM, Bowen WH. Binding properties of streptococcal glucosyltransferases for hydroxyapatite, saliva-coated hydroxyapatite, and bacterial surfaces. Arch Oral Biol 1998; 43: 103–10.
12. Rolla G, Ciardi JE, Schultz SA. Adsorption of glucosyltransferase to saliva coated hydroxyapatite. Possible mechanism for sucrose dependent bacterial colonization of teeth. Scand J Dent Res 1983; 91: 112–17.
13. Vacca-Smith AM, Venkitaraman AR, Quivey RG, Jr., Bowen WH. Interactions of streptococcal glucosyltransferases with alpha-amylase and starch on the surface of saliva-coated hydroxyapatite. Arch Oral Biol 1996; 41: 291–8.
14. Fukui K, Moriyama T. Effect of maltose on glucan synthesis by glucosyltransferases of *Streptococcus mutans*. Microbiol Immunol 1983; 27: 917–27.
15. Fu DT, Robyt JF. Maltodextrin acceptor reactions of *Streptococcus mutans* 6715 glucosyltransferases. Carbohydr Res 1991; 217: 201–11.
16. McCabe MM, Hamelik RM. An enzyme from *Streptococcus mutans* forms branches on dextran in the absence of sucrose. Biochem Biophys Res Commun 1983; 115: 287–94.
17. Russell RR, Shiroza T, Kuramitsu HK, Ferretti JJ. Homology of glucosyltransferase gene and protein sequences from *Streptococcus sobrinus* and *Streptococcus mutans*. J Dent Res 1988; 67: 543–7.
18. Russell RR. The application of molecular genetics to the microbiology of dental caries. Caries Res 1994; 28: 69–82.
19. Ueda S, Shiroza T, Kuramitsu HK. Sequence analysis of the *gtfC* gene from *Streptococcus mutans* GS-5. Gene 1988; 69: 101–9.
20. Monchois V, Arguello-Morales M, Russell RR. Isolation of an active catalytic core of *Streptococcus downei* MFe28 GTF-I glucosyltransferase. J Bacteriol 1999; 181: 2290–2.
21. Kralj S, van Geel-Schutten GH, Dondorff MM, Kirsanovs S, van der Maarel MJ, Dijkhuizen L. Glucan synthesis in the genus Lactobacillus: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains. Microbiology 2004; 150: 3681–90.
22. Devulapalle KS, Mooser G. Subsite specificity of divalent metal ions to glucosyltransferase. J Craniofac Genet Dev Biol 2000; 20: 107–8.
23. Ito K, Ito S, Shimamura T, Weyand S, Kawarasaki Y, Misaka T, et al. Crystal structure of glucansucrase from the dental caries pathogen *Streptococcus mutans*. J Mol Biol 2011; 408: 177–86.
24. Wunder D, Bowen WH. Action of agents on glucosyltransferases from *Streptococcus mutans* in solution and adsorbed to experimental pellicle. Arch Oral Biol 1999; 44: 203–14.
25. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov 2005; 4: 206–20.
26. Ooshima T, Minami T, Aono W, Izumitani A, Sobue S, Fujiwara T, et al. Oolong tea polyphenols inhibit experimental dental caries in SPF rats infected with mutans streptococci. Caries Res 1993; 27: 124–9.
27. Touyz LZ, Amsel R. Anticariogenic effects of black tea (*Camellia sinensis*) in caries prone-rats. Quintessence Int 2001; 32: 647–50.
28. Linke HA, LeGeros RZ. Black tea extract and dental caries formation in hamsters. Int J Food Sci Nutr 2003; 54: 89–95.

29. Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Res 1991; 25: 438–43.
30. Hattori M, Kusumoto IT, Namba T, Ishigami T, Hara Y. Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. Chem Pharm Bull (Tokyo) 1990; 38: 717–20.
31. Nakahara K, Kawabata S, Ono H, Ogura K, Tanaka T, Ooshima T, et al. Inhibitory effect of oolong tea polyphenols on glycosyltransferases of mutans Streptococci. Appl Environ Microbiol 1993; 59: 968–73.
32. Hirasawa M, Takada K, Otake S. Inhibition of acid production in dental plaque bacteria by green tea catechins. Caries Res 2006; 40: 265–70.
33. Ooshima T, Minami T, Aono W, Tamura Y, Hamada S. Reduction of dental plaque deposition in humans by oolong tea extract. Caries Res 1994; 28: 146–9.
34. Matsumoto M, Minami T, Sasaki H, Sobue S, Hamada S, Ooshima T. Inhibitory effects of oolong tea extract on caries-inducing properties of mutans streptococci. Caries Res 1999; 33: 441–5.
35. Sasaki H, Matsumoto M, Tanaka T, Maeda M, Nakai M, Hamada S, et al. Antibacterial activity of polyphenol components in oolong tea extract against *Streptococcus mutans*. Caries Res 2004; 38: 2–8.
36. Koo H, Rosalen PL, Cury JA, Park YK, Bowen WH. Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity. Antimicrob Agents Chemother 2002; 46: 1302–9.
37. Koo H, Pearson SK, Scott-Anne K, Abranches J, Cury JA, Rosalen PL, et al. Effects of apigenin and tt-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats. Oral Microbiol Immunol 2002; 17: 337–43.
38. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. Caries Res 1991; 25: 347–51.
39. Koo H, Vacca Smith AM, Bowen WH, Rosalen PL, Cury JA, Park YK. Effects of Apis mellifera propolis on the activities of streptococcal glucosyltransferases in solution and adsorbed onto saliva-coated hydroxyapatite. Caries Res 2000; 34: 418–26.
40. Koo H, Hayacibara MF, Schobel BD, Cury JA, Rosalen PL, Park YK, et al. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. J Antimicrob Chemother 2003; 52: 782–9.
41. Koo H, Schobel B, Scott-Anne K, Watson G, Bowen WH, Cury JA, et al. Apigenin and tt-farnesol with fluoride effects on *S. mutans* biofilms and dental caries. J Dent Res 2005; 84: 1016–20.
42. Gregoire S, Singh AP, Vorsa N, Koo H. Influence of cranberry phenolics on glucan synthesis by glucosyltransferases and *Streptococcus mutans* acidogenicity. J Appl Microbiol 2007; 103: 1960–8.
43. Koo H, Duarte S, Murata RM, Scott-Anne K, Gregoire S, Watson GE, et al. Influence of cranberry proanthocyanidins on formation of biofilms by *Streptococcus mutans* on saliva-coated apatitic surface and on dental caries development *in vivo*. Caries Res 2010; 44: 116–26.
44. Yanagida A, Kanda T, Tanabe M, Matsudaira F, Oliveira Cordeiro JG. Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of mutans streptococci. J Agric Food Chem 2000; 48: 5666–71.
45. Steinberg D, Feldman M, Ofek I, Weiss EI. Effect of a high-molecular-weight component of cranberry on constituents of dental biofilm. J Antimicrob Chemother 2004; 54: 86–9.
46. Yamanaka A, Kimizuka R, Kato T, Okuda K. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiol Immunol 2004; 19: 150–4.
47. Duarte S, Gregoire S, Singh AP, Vorsa N, Schaich K, Bowen WH, et al. Inhibitory effects of cranberry polyphenols on formation and acidogenicity of *Streptococcus mutans* biofilms. FEMS Microbiol Lett 2006; 257: 50–6.
48. Matsumoto M, Hamada S, Ooshima T. Molecular analysis of the inhibitory effects of oolong tea polyphenols on glucan-binding domain of recombinant glucosyltransferases from *Streptococcus mutans* MT8148. FEMS Microbiol Lett 2003; 228: 73–80.
49. Osawa K, Miyazaki K, Shimura S, Okuda J, Matsumoto M, Ooshima T. Identification of cariostatic substances in the cacao bean husk: their anti-glucosyltransferase and antibacterial activities. J Dent Res 2001; 80: 2000–4.
50. Hamada S, Kontani M, Hosono H, Ono H, Tanaka T, Ooshima T, et al. Peroxidase-catalyzed generation of catechin oligomers that inhibit glucosyltransferase from *Streptococcus sobrinus*. FEMS Microbiol Lett 1996; 143: 35–40.
51. Murata RM, Branco de Almeida LS, Yatsuda R, Dos Santos MH, Nagem TJ, Rosalen PL, et al. Inhibitory effects of 7-epiclusianone on glucan synthesis, acidogenicity and biofilm formation by *Streptococcus mutans*. FEMS Microbiol Lett 2008; 282: 174–81.
52. Wolinsky LE, Mania S, Nachnani S, Ling S. The inhibiting effect of aqueous Azadirachta indica (Neem) extract upon bacterial properties influencing *in vitro* plaque formation. J Dent Res 1996; 75: 816–22.
53. Wu-Yuan CD, Chen CY, Wu RT. Gallotannins inhibit growth, water-insoluble glucan synthesis, and aggregation of mutans streptococci. J Dent Res 1988; 67: 51–5.
54. Ciardi JE, Bowen WH, Rolla G. The effect of antibacterial compounds on glucosyltransferase activity from *Streptococcus mutans*. Arch Oral Biol 1978; 23: 301–5.
55. Devulapalle KS, Mooser G. Subsite specificity of the active site of glucosyltransferases from *Streptococcus sobrinus*. J Biol Chem 1994; 269: 11967–71.
56. Wright WG, Thelwell C, Svensson B, Russell RR. Inhibition of catalytic and glucan-binding activities of a streptococcal GTF forming insoluble glucans. Caries Res 2002; 36: 353–9.
57. Newbrun E, Hoover CI, Walker GJ. Inhibition by acarbose, nojirimycin and 1-deoxynojirimycin of glucosyltransferase produced by oral streptococci. Arch Oral Biol 1983; 28: 531–6.
58. Ren Z, Cui T, Zeng J, Chen L, Zhang W, Xu X, et al. Molecule targeting Glucosyltransferase Inhibits *Streptococcus mutans* biofilm formation and virulence. Antimicrob Agents Chemother 2015; 60: 126–35.
59. Mesleh MF, Rajaratnam P, Conrad M, Chandrasekaran V, Liu CM, Pandya BA, et al. Targeting bacterial cell wall peptidoglycan synthesis by inhibition of glycosyltransferase activity. Chem Biol Drug Des 2016; 87: 190–9.
60. Devulapalle KS, Mooser G. Glucosyltransferase inactivation reduces dental caries. J Dent Res 2001; 80: 466–9.
61. Culshaw S, Larosa K, Tolani H, Han X, Eastcott JW, Smith DJ, et al. Immunogenic and protective potential of mutans streptococcal glucosyltransferase peptide constructs selected by major histocompatibility complex class II allele binding. Infect Immun 2007; 75: 915–23.
62. Olson GA, Guggenheim B, Small PA, Jr. Antibody-mediated inhibition of dextran-sucrose-induced agglutination of *Streptococcus mutans*. Infect Immun 1974; 9: 273–8.
63. Douglas CW, Russell RR. Effect of specific antisera on adherence properties of the oral bacterium *Streptococcus mutans*. Arch Oral Biol 1982; 27: 1039–45.
64. Kawato T, Yamashita Y, Katono T, Kimura A, Maeno M. Effects of antibodies against a fusion protein consisting of parts of cell surface protein antigen and glucosyltransferase of

Streptococcus sobrinus on cell adhesion of mutans streptococci. Oral Microbiol Immunol 2008; 23: 14–20.
65. Oho T, Shimazaki Y, Mitoma M, Yoshimura M, Yamashita Y, Okano K, et al. Bovine milk antibodies against cell surface protein antigen PAc-glucosyltransferase fusion protein suppress cell adhesion and alter glucan synthesis of *Streptococcus mutans*. J Nutr 1999; 129: 1836–41.
66. Wunder D, Bowen WH. Effects of antibodies to glucosyltransferase on soluble and insolubilized enzymes. Oral Dis 2000; 6: 289–96.
67. Kopec LK, Vacca Smith AM, Wunder D, Ng-Evans L, Bowen WH. Influence of antibody on the structure of glucans. Caries Res 2002; 36: 108–15.
68. Hamada S, Horikoshi T, Minami T, Kawabata S, Hiraoka J, Fujiwara T, et al. Oral passive immunization against dental caries in rats by use of hen egg yolk antibodies specific for cell-associated glucosyltransferase of *Streptococcus mutans*. Infect Immun 1991; 59: 4161–7.
69. Kruger C, Pearson SK, Kodama Y, Vacca Smith A, Bowen WH, Hammarstrom L. The effects of egg-derived antibodies to glucosyltransferases on dental caries in rats. Caries Res 2004; 38: 9–14.
70. Mitoma M, Oho T, Michibata N, Okano K, Nakano Y, Fukuyama M, et al. Passive immunization with bovine milk containing antibodies to a cell surface protein antigen-glucosyltransferase fusion protein protects rats against dental caries. Infect Immun 2002; 70: 2721–4.
71. Bowen WH. Do we need to be concerned about dental caries in the coming millennium? Crit Rev Oral Biol Med 2002; 13: 126–31.
72. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res 2011; 90: 294–303.
73. Tamesada M, Kawabata S, Fujiwara T, Hamada S. Synergistic effects of streptococcal glucosyltransferases on adhesive biofilm formation. J Dent Res 2004; 83: 874–9.
74. Reese S, Guggenheim B. A novel TEM contrasting technique for extracellular polysaccharides in in vitro biofilms. Microsc Res Tech 2007; 70: 816–22.