© Med Sci Monit, 2007; 13(11): RA196-203
PMID: 17968308

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Review Article

Received: 2007.05.11
Accepted: 2007.09.15
Published: 2007.11.01

Dental caries: From infection to prevention

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Source of support: Self financing

Summary

Dental caries is one of the most prevalent diseases in humans, second only to the common cold. It causes irreversible damage to the grinding machinery involved in the intake of food and hence causes great distress. The changes in the homeostasis of the oral cavity with an overgrowth of *Streptococcus mutans* is recognized as the primary cause of the disease. Most treatments are now aimed at either elimination of this bacterium or suppression of its virulence. *S. mutans* strongly adheres and releases acids by the fermentation of carbohydrates, leading to the demineralization of the tooth. This attachment is mediated mostly by the interaction of surface proteins and bacterial polysaccharides. Ambiguities in the basic treatment of dental caries, such as the use of fluoride and antibiotics, vitalize the deployment of probiotic therapies for its cure. The growing research in herbal treatments has led to the discovery of various phytochemicals to limit the virulence of *S. mutans*. This review focuses on the properties of *S. mutans* in cariogenicity and outlines ways to combat dental caries.

key words: **dental caries • *S. mutans* • cariogenic**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=512939

Word count: 3657
Tables: –
Figures: 3
References: 107

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Background

Dental caries is the predominant cause of tooth decay. Although the affliction is not life threatening, it causes nagging pain and thus possess physical as well as psychological discomfort. The economic burden of the disease is also very high. If treatment were available, the costs of dental caries alone would exceed the total healthcare budget for children in many low-income countries. The earliest detectable stage is the appearance of a white spot on the tooth. This may later advance in steps to form a lesion, cavitations, and then to complete tooth loss. The cavities caused in dental caries are usually painless until they damage the vascular system of the tooth. Sweets often cause cavities and dentists fill them. However, whether caries is the natural fate of the tooth or there is a particular preventable factor that leads to it has been a topic of intense debate. Nevertheless, it is clear that the condition is complex and multifactorial.

Dental Caries as an Infection

Is dental caries infectious?

The microbiological study on human dental plaque dates back to 1924 when Clarke first observed oral Streptococci [1]. The infectious and transmittable nature of dental caries was brought in focus by the studies by Keyes on gnotobiotic rodents in 1960. He found that germ-free hamsters developed caries when they were caged together with caries-active hamsters. He discovered that certain Streptococci, later identified as *Streptococcus mutans*, were the main causative agent of dental caries [2]. This brought an upsurge in the microbiology of plaque. In addition, numerous epidemiological data suggest the infectious nature of dental caries in humans [3].

Role of *Streptococcus mutans* in dental caries

Human dental plaque harbors nearly 200–300 species [4]. Increasing knowledge from research in this area indicates that the oral cavity is a complex ecosystem with highly diverse acid-tolerant and acid-producing microbiota. The fermentation of carbohydrates by acidogenic oral microorganisms is the key factor in the development of dental caries. Carious lesions result primarily from the dissolution of mineral in enamel and dentin due to acids released by these microbes [5]. The primary acid-tolerant bacteria associated with the plaque are *S. mutans*, *S. anginosus*, *S. constellatus*, *S. gordonii*, *S. intermedius*, *S. mitis*, *S. oralis*, *S. salivarius*, and *S. sanguis* [6].

Mutans streptococci are the most cariogenic pathogens as they are highly acidogenic, producing short-chain carboxylic acids which dissolve hard tissues such as enamel and dentine [7]. In addition, they ferment sucrose and produce insoluble extracellular polysaccharides, which enhance their adherence to the tooth surface and encourage biofilm formation [8]. The two species of *mutans* streptococci most commonly isolated from tooth samples are *S. mutans* and *S. sobrinus*. *S. mutans* is more cariogenic than *S. sobrinus* because specific cell-surface proteins of *S. mutans* aid in its primary attachment to the tooth, while *S. sobrinus* lacks such proteins [9]. The occurrence of both species together makes the oral environment more conducive to caries [10]. Colonization of tooth by *mutans* Streptococci occurs soon after tooth eruption. If followed by the colonization of fissures in their depths, this inevitably leads to caries [9].

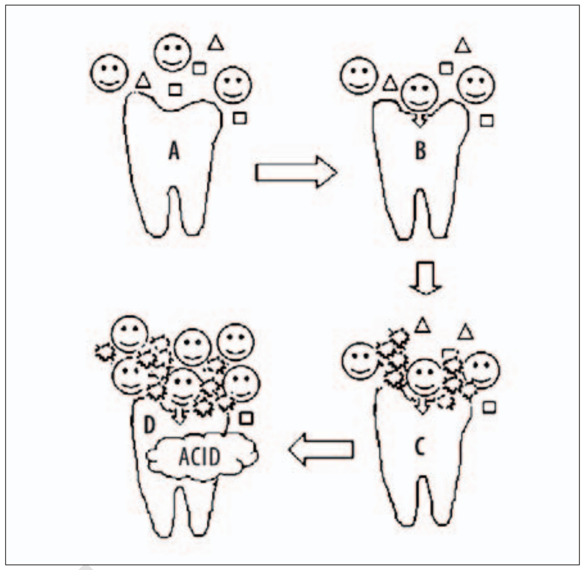

Figure 1. Stages leading to colonization and infection by *S. mutans* on the tooth surface. A – normal oral flora. B – Initial adhesin-mediated attachment of *S. mutans*. C – Synthesis of extracellular polysaccharide by bacteria resulting in aggregation of *S. mutans*. D – The acid released by aggregated cells leads to demineralization and cavitation of tooth. *S. mutans* and other oral microbes – ☺, △, □, adhesin – ⇩, Sucrose moeity – .

Virulence factors of *Streptococcus mutans*

Streptococcus mutans is a member of indigenous oral microflora. What makes this bacterium virulent and cariogenic is a subject of keen interest (Figure 1). *S. mutans*, being a facultative anaerobe, can survive anywhere in the oral cavity. It can ferment most of the sugars and sugar alcohols present in food such as glucose, sucrose, lactose, trehalose, mannitol, sorbitol, raffinose, and melibiose [11].

Initial and polysaccharide-mediated adherence

The attachment of bacteria to the host tissue is the preliminary step for infection and colonization. Initial adherence of *S. mutans* to the tooth is mediated by cell-surface adhesin-like proteins [12]. The most extensively studied and characterized one is the 180-210 kDa protein present on *S. mutans'* cell surface. This protein is designated by various names, such as PAc, antigen I/II, P1, and Spa P1. Not only does it mediate the adhesion of the bacterium to the tooth, but it also provides the sites for further attachment [13]. Recent findings indicate a reduction in the virulence of *S. mutans* on suppression of this protein [14].

Streptococcus mutans metabolizes sucrose to synthesize water-soluble and/or insoluble glucans. The reactions are catalyzed by three isozymes of glucosyltransferases (GTFs) (E.C. 2.4.1.5), i.e. GTF-B, GTF-C, and GTF-D. The molecular weight of GTF ranges from 150 to 180 kDa. These enzymes catalyze the transfer and addition of a glucosyl moi-

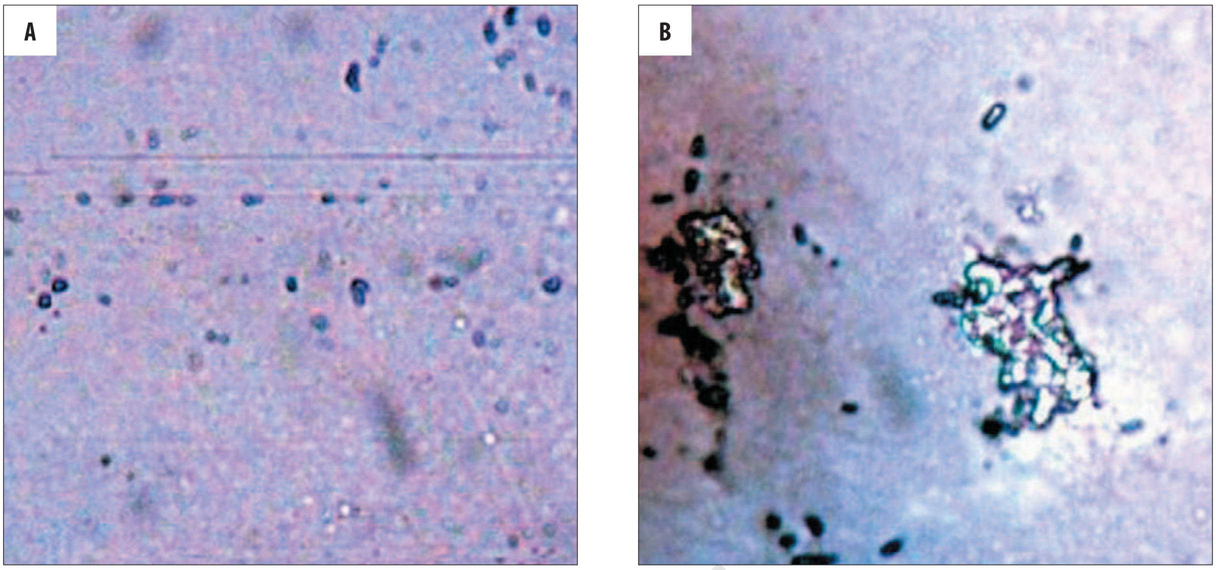

Figure 2. Microscopic slides (40×) depicting biofilm formation of *S. mutans*. (**A**) *S. mutans* in the absence of glucan. Slide showing unaggregated cells. (**B**) Glucan-mediated clumping of cell leading to the biofilm formation.

ety to the terminal site of a primer or elongating glucan [15] according to the reaction scheme:

$$\text{sucrose}+(1,6\text{-}\alpha\text{-D-glucosyl})_n \rightarrow \text{D-fructose}+(1,6\text{-}\alpha\text{-D-glucosyl})_{n+1}$$

The glucans consist of a α-(1-6)-linked glucose polymer with α-(1-3) branch linkages [16]. The sticky nature of glucan facilitates the adherence of bacteria to the tooth and resists its detachment by normal mechanical forces such as mastication, swallowing, and chewing inside the mouth. Also, *S. mutans* produces three glucan-binding proteins (Gbps), GbpA, GbpB, and GbpC. The role of these proteins in mediating sucrose-dependent adherence has been proven and established [17].

In addition to glucan synthesis, bacteria use fructosyltransferases to synthesize fructans with β-(2-1)-D-fructo-furanosidic linkages with average repeating units of 8 and 27 sugar residues [18]. Fructans are believed to function exclusively as extracellular storage reservoirs, but a few reports implicate their role in plaque formation as well [19,20]. Since both GTFs and FTFs utilize the same substrate, i.e. sucrose, its role in cellular adherence and caries induction cannot be ignored.

Biofilm lifestyle

The formation of dental plaque is a multistep mechanism. Initial attachment of *S. mutans* is followed by its accumulation and proliferation, leading to the formation of a sessile, exopolymer-shrouded community known as a biofilm (Figure 2) [21]. Biofilms can tolerate numerous adverse conditions such as antimicrobial agents, variation in pH, and nutrient and oxygen deprivation. The physiology of the organism in such surface-associated communities is different from that of planktonic cells [22]. The members of a biofilm confer a reproductive fitness as they have a reduced rate of growth relative to their planktonic siblings [23]. Dental biofilm formation occurs through a series of stages. The first stage involves deposition of an acquired enamel pellicle (AEP) on the tooth. This acellular coating (AEP) includes salivary components and bacterial constituents such as bacterial GTFs and FTF which synthesize exopolysaccharides. The second stage involves the adherence and co-adherence of bacteria from the oral cavity with the aid of polysaccharides on the AEP. Proliferation occurs in the third stage, while in the final stage the biofilm reaches a steady state in relation to the surrounding environment [23]. All these steps involve the concerted action of a set of genes [24]. Characterization of the corresponding hypothetical proteins is underway [25].

Aciduricity and acidogenicity

Streptococcus mutans can both produce and tolerate acid to help its survival in the oral cavity. *S. mutans* is reported to be a homofermentative lactic acid bacterium [26,27], but when the carbohydrate supply is limited, this bacterium also produce formate, acetate, and ethanol. A considerable decrease from pH 7.0 was observed in the oral cavity within a few minutes after a glucose rinse [28]. This implies that the bacterium is rapidly acidogenic. This is mediated by a highly efficient phosphotransferase system for glucose and sucrose [29]. The aciduricity of *S. mutans* adds to its cariogenic potential. This is achieved by upregulation of a proton-translocating F_1-F_0 ATPase that extrudes H^+ from the cell [30]. The protein expression in this process resembles other stress-induced responses, such as salt, heat, starvation, and oxidative stress [31].

Intracellular polysaccharides

In the presence of extracellular glucose and sucrose, *Streptococcus mutans* synthesizes intracellular glycogen-like polysaccharides (IPSs). These IPSs are similar to those of other oral streptococci and are glucose homopolymers with α-(1-4) and α-(1-6) linkages [32]. The synthesis of IPS is linearly proportional to the extracellular carbohydrate concentration (glucose or sucrose) [33]. The metabolism of IPS may foster the development of caries by prolonging the exposure time to organic acids when the bacterium is devoid of an external food source. Numerous reports confirm IPS as an important contributor to the cariogenicity of *Streptococcus mutans* [34].

Mutacins

Bacteriocins are proteinaceous antibacterial substances produced by some bacteria to inhibit or interfere with the growth of other bacteria. *Streptococcus mutans* produces bacteriocin, mutacin, which is active against other streptococcal species and non-streptococcal Gram-positive bacteria [35]. The production of the bacteriocin helps in the efficient establishment and hence colonization of this bacterium inside the oral cavity [36]. Hillman et al. found that bacterial strains with increased mutacin production could colonize the oral flora of an adult even after a single application [37]. The stability of such a plaque ecosystem, in addition to its microbial composition, is also an important factor [38]. Several mutacins have been purified and characterized biochemically [39].

Remedies for Dental Caries

Habits and hygiene

In spite of the different causes sought and discovered, the primary cause of dental caries is still negligence in oral hygiene. The oral cavity of a newborn is essentially free of all microbes. Soon after the birth, numerous bacteria, including *Streptococcus mutans*, start developing niches for themselves. Infants with prevalent and frequent bottle or breast feeding are prone to extensive decay of maxillary anterior teeth, called nursing caries [40]. As the saliva flow during sleep is reduced, bacteria gain prolonged access to fermentable substances such as sucrose or lactose and cause the decay.

Dental caries is correlated with sugar uptake in the diet. The increase in urbanization has led to the replacement of crude sugar from natural sources by refined sugar, which worsened the situation [41]. Numerous studies indicate a linear correlation of sugar consumption with dental caries in populations worldwide [42,43]. However, starchy foods and fresh fruit are reported to be less cariogenic. Foods that involve extensive mastication stimulate the production of saliva and hence have a low cariogenic potential. Fibrillar and firm fruit such as apples and carrots act as natural toothbrushes, as they clean the tooth surface during chewing. Substitution of fermentable carbohydrates by xylilitol, saccharin, and aspartame has been efficiently used for reducing caries [44]. However, long-term use of artificial sweeteners is unadvisable due to their probable role as carcinogens [45,46]. Fluids such as juices and milk seem to be less cariogenic as they are not retained as much in the oral cavity. Consumption of carbonated drinks, especially by children, is a predominant cause of dental caries and should be strictly discouraged [47].

Oral care, undoubtedly, begins with oral hygiene. Frequent brushing and flossing of teeth helps remove bacteria and fermentable substances. The continuous flow of saliva reduces the cariogenic flora on the tooth. Saliva also acts as a buffering agent during the continuous acid production in the oral cavity [28]. Individuals with dry mouth syndrome are hence more prone to dental caries. This has been observed with people following radiation treatment of head and neck cancers, in narcotics users, and in patients with Sjögren's syndrome [48,49].

Fluoride uptake

The enamel and dentin of our teeth are comprised of minerals, mainly carbonated hydroxyapatite, and can be approximately represented by the formula:

$$Ca_{10-x}(Na)_x(PO_4)_{6-y}(CO_3)_z(OH)_{2-u}(F)_u \text{ [50].}$$

Dental caries is essentially a disease of demineralization. Considerable data support fluoride uptake as a remedy to demineralization by acting as an active agent in remineralization. The electrostatic interaction between Ca^{+2} and the F^- is greater than that between Ca^{+2} and OH^- ions, making the fluoridated apatite lattice more crystalline and more stable [51]. As a consequence, it becomes less soluble in acid [52]. The direct topical administration of fluoride on the tooth after its eruption appears more effective than its intake in the diet [53]. It is therefore the most important component in toothpastes and mouthwashes.

Fluorides may also inhibit bacterial metabolism. Fluorine in the ionic form (F^-) cannot cross the cell wall and membrane of bacteria, but can be taken up readily as HF [54]. When the pH in the plaque falls, a portion of the F^- ions combines with hydrogen ions to from HF and diffuses into the cell. After entry into the cell, HF acidifies the cell and dissociates, thereby releasing F^- ions. F^- ions are toxic to cells as they interfere with the enzyme machinery of the cell [54]. Although the most common first line of defense, no absolute inverse relationship between fluoride-uptake and caries could be observed universally yet [55,56].

Antibiotics

Ever since the establishment of dental caries as an infectious disease, antibiotics are constantly in use for treating it. Chlorhexidine digluconate (CHX) had been a gold standard in this field. It has been used with the aim of elimination or suppression of *mutans* streptococci in the oral cavity. CHX damages outer cell layers, but is insufficient in inducing lysis or death [57]. It then crosses the cell wall or outer membrane by passive diffusion and attacks the bacterial cytoplasmic membrane [58]. This is followed by leakage of the cellular constituents. A high concentration of CHX causes coagulation of the intracellular constituents. As a result, the cytoplasm becomes congealed, with a consequent reduction in leakage. Thus there is a biphasic effect on membrane permeabilities: in the presence of CHX, a high rate of leakage is observed initially, but as CHX concentration increases, leakage decreases because of the coagulation of the cytosol [58]. The best results in reducing dental plaque have been observed with chlorhexidine gels and mouthwashes [59]. As disparities in its efficacy against varying subjects have been observed, CHX cannot be regarded as the single line of defense for the cure of caries [60].

The pioneering work of McClure and Hewitt indicates the usefulness of penicillin in preventing experimentally in-

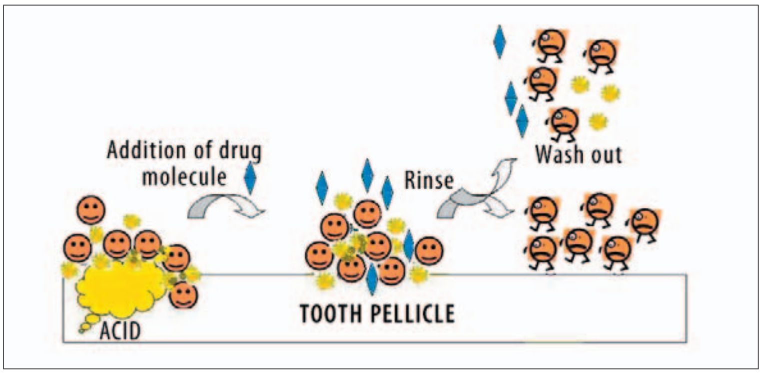

Figure 3. An ideal approach to the control of dental caries. *S. mutans* – / . *S. mutans* in oral cavity aggregated on the surface of teeth and ferment sugars to produce acid leading to pathogenicity. Sucrose moiety – . Sucrose moieties facilitate aggregation of bacterial cells on the tooth surface. Drug candidate – . A potent drug combating pathogens to reduce the cariogenicity. The drug should specifically interact with *S. mutans* and inhibit both adhesin and polysaccharide mediated attachment. It should act as a buffering agent and control the acid production by oral microbes.

duced caries [61]. Many other antibiotics with activity against Gram-positive bacteria depress the development of dental caries in experimental animals [62,63]. *S. mutans* has been reported to be highly susceptible to penicillin, methicillin, ampicillin, erythromycin, cephalothin, and many other antibiotics [64]. As these antibiotics are active only against planktonic bacteria, more antibiotics are now screened for their effects against oral bacteria [65].

Rediscovering traditional medicines: Herbal cure

Various chewing sticks have been in use since old times. Due to the lower occurrence of caries in people using them, their mode of action is being explored. Extracts from the roots and stems of *Salvadora persica* have been used for the treatment of oral infections in animals [66]. Aqueous and ethanolic extracts of *S. persica* proved useful in removing the smear layer from dentin surfaces [67]. It had been found that aqueous extracts of *S. persica* bark, pulp, as well as the whole of it were effective against various bacteria, including *Streptococcus mutans* [68]. The chewing sticks used in India are usually from *Azadiracta indica* (Neem). Neem extracts show antimicrobial effects against *Streptococcus mutans* and *S. faecalis* [69]. A formulation of mucoadhesive dental gel containing Neem leaves extract (25 mg/g) reduced both the plaque index and bacterial count [70]. Acacia is also used as an active constituent in toothpastes in India. The components of its bark and gum, mainly tannins, show antimicrobial and astringent effects [71]. Plant tannins have the ability to reduce the attachment of *S. mutans* by binding to proline rich protein of the salivary pellicle or to the cell-surface lipoteichoic acid [72].

As most of the commonly used antibiotics are effective against planktonic bacteria, more studies are now aimed at targeting biofilms of *S. mutans* as a whole [73]. Notably, glucan-mediated bacterial attachment is an important feature of biofilm formation and a plethora of herbs are being evaluated for this effect [74–76]. Treatments with extracts of *Andrographis paniculata*, *Cassia alata*, Chinese black tea, guava, and *Harrisonia perforale* show decreases in the adherence of *S. mutans* to a glass surface as well as saliva-coated hydroxyapatite beads [77]. Very recently, specific compounds from guava have been characterized for their anticariogenic potential [78]. Oolong tea extracts show remarkable inhibitory effect on the rate of bacterial acid production along with retardation in growth of *S. mutans*. This has been attributed to the polyphenols present in the extracts that also reduce the cell surface hydrophobicity of the bacterium [79]. Many essential oils in combination with CHX have been tested for better efficacy in inhibiting growth as well as biofilms of oral flora. Essential oils from cinnamon, *Leptospermum morrisoni*, manuka, and tea tree oil exhibited growth inhibitory effects against cariogenic bacteria. They suppress biofilm formation during planktonic growth as well as growth of preformed biofilms [80]. Chlorhexidine in combination with cinnamon, *L. morrisoni*, and manuka oil resulted in better efficiency of chlorhexidine in inhibiting the growth of *S. mutans* [80].

Compounds found in propolis affect the growth and glucosyltransferase activity of *S. mutans* [81]. Topical application of propolis twice a day or its inclusion in drinking water reduced the incidence of dental caries in rats [82]. Of the various components of propolis studied earlier, tt-farnesol was the most effective antibacterial agent, while apigenin was found to be a potent inhibitor of glucosyltransferase [83].

Probiotic therapies

Great progress has been made in developing a cure for dental caries with the help of genetic engineering [84]. The sequencing of the complete *S. mutans* genome marks the beginning of an era of newer therapies [85]. An ideal drug for caries prevention should aim at maintaining the normal homeostasis of the oral cavity and reducing the virulence of *S. mutans* (Figure 3).

Vaccines

Immune defense in dental caries is mediated mainly by secretory IgA (sIgA) antibodies present in saliva and generated by the mucosal immune system. The mode of action of these antibodies is inhibition of the adherence and possibly metabolic activities of *S. mutans* [86]. In view of vaccine development against caries, the virulence factors, specifically the adherence-motivating factors, are recognized as key antigens. The research focus is mainly on the incorporation of these antigens into mucosal immune systems and delivery with or without adjuvants to mucosal IgA-inductive sites [87]. Novel strategies using antigen I/II, glucosyltransferases and glucan binding proteins are designed for pursuing vaccine goals as these are the main proteins that mediate the attachment of bacteria. Induction of salivary IgA and circulating IgG antibodies have been observed by oral or intranasal immunization with these antigens in many animal models [88]. Similar antigen preparations in human trials have shown successful induction of salivary sIgA [89,90]. Tailored vaccines possessing immunogenic sites of the virulence determinant traits have shown significant caries-preventive results [91]. Chimeric proteins incorporating both AgI/II and GTF epitopes have shown most encouraging responses [92]. DNA vaccines based on these proteins are also being developed and assayed for their anticariogenic activity [93]. Better efficiency of a fusion DNA vaccine coding for both AgI/II and glucosyltransferases in rats has also been reported [84]. Numerous approaches for passive immunization are employed to avoid complications that might arise from active immunization [94]. Immunity through antibodies for glucosyltransferases available from cow's milk [95], hen's eggs [96,97], and plantibodies [98,99] has been reported. Efficient delivery systems are being developed for a continuous and controlled release of vaccines. Both animated [100] and non-animated vehicles such as liposomes [101] and microparticles [102] have been assessed in animal models.

Replacement therapy

In the post-genomic era, recombinant DNA technology is being used to find an answer to dental caries. One of the promising developments is replacement therapy. Genetic engineering is being used to tailor the effector strain for replacement therapy of dental caries, which acts as a vaccine and should not be pathogenic. Moreover, it colonizes the niche, thereby preventing colonization and outgrowth of wild-type strains. Using this approach, a harmless strain is permanently implanted in the host's oral flora. Once established, the effector strain competes with the wild-type strain and prevents its outgrowth [103].

An effector strain with such properties, BCS3-L1, was constructed using a clinical isolate, JH1 140, with high mutacin activity and genetic stability [104]. The mutagenesis approach has also been used successfully by constructing a lactate dehydrogenase mutant which has been reported to reduce cariogenic potential [105]. The resultant strain thus produced was deficient in lactic acid production and had elevated mutacin levels. Colonization studies with the mutant strain BCS3-L1 shows no pathogenic hazards: histopathological examination shows no detectable carious lesions [106]. Oragenics (www.oragenics.com) is the company that is ready with the first biotic-mouthwash, although the therapy still awaits its launch after remaining trials and final approval.

Conclusions

Dental caries is a disease that can usually be successfully prevented or controlled. It is an important task for the dental team to teach individuals to take correct actions to minimize the risk for the disease. It is also possible to identify and evaluate factors of importance for cavity formation. By targeted actions, such risk factors can usually be minimized, resulting in a reduced risk for caries. Due to the fact that dental caries is a multifactorial disease, a number of methods exist to prevent it. These apply mainly to patients in industrialized countries, although the basic principles are the same world-wide. Caries can be reduced by eliminating *Streptococcus mutans* populations from the oral cavity, increasing the acid-resistance of teeth, and control of the carbohydrate composition of the diet. Apparently safe and efficacious oral anti-mutant vaccines have been developed in the laboratory. Application of glucan-hydrolyzing enzymes to prevent the attachment of *Streptococcus mutans* to hard surfaces has been evaluated, but so far with little success [107]. Animal studies suggest that there is great promise in the implantation of benign oral microbial strains capable of successfully completing with *Streptococcus mutans* (replacement therapy), but few human trials have been undertaken to date. Mechanical methods of plaque control, including brushing, flossing, and professional scaling, are only temporarily effective in eliminating *Streptococcus mutans*. The control of plaque growth by chemical means has attracted considerable attention and chlorhexidine has been shown to be effective, although it causes discoloration of the teeth upon prolonged use. Fluoride is still the best available anti-caries chemical agent; its anti-caries action is attributed to increasing the resistance of the tooth to acid demineralization, stimulation of remineralization, and inhibition of *S. mutans'* carbohydrate metabolism. Although sugar substitutes could potentially play an important role in caries control, consumer preference continues to overwhelmingly favor the use of sucrose in food products.

References:

1. Clarke JK: On the bacterial factor in the aetiology of dental caries. Br J Exp Pathol, 1924; 5: 141–47
2. Fitzgerald RJ, Keyes PH: Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J Am Dent Assoc, 1960; 61: 9–18
3. Beck J, Drake C: Some epidemiologic evidence on the etiology of caries. Community Dent Oral Epidemiol, 1975; 3(5): 223–27
4. Hamilton IR, Bowden GH: Oral microbiology. In: Encyclopedia of Microbiology Lederberg J (ed.). Academic press, San Diego, CA. 2000; 466–80
5. Arends J, Christoffersen J: The nature of early caries lesions in enamel. J Dent Res, 1986; 65: 2–11
6. Svensater S, Borgstrom M, Bowden GHW, Edwardsson S: The Acid-tolerant microbiota associated with plaque from initial caries and healthy tooth surfaces. Caries Res, 2003; 37: 395–403
7. Shaw JH: Causes and Control of dental caries. N Engl J Med, 1987; 317(16): 996–1004
8. Shen S, Samarnayake LP, Yip HK: *In-vitro* growth, acidogenicity and cariogenicity of predominant human root caries flora. J Dent, 2004; 37: 667–78
9. Loesche WJ: Role of *Streptococcus mutans* in human dental decay. Bacteriological reviews, 1986; 50(4): 353–80
10. Okada M, Soda Y, Hayashi F et al: Longitudinal studies on dental caries incidence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in pre-school children. J. Med Microbiol, 2005; 54: 661–65
11. Coykendall AL: Classification and identification of the *Viridans streptococci*. Clinical Microbiology Reviews, 1989; 2(3): 315–28
12. Russell MW, Lehner T: Characterization of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype C. Arch Oral Biol, 1978; 23(1): 7–15
13. Jenkinson HF, Demuth DR: Structure, function and immunogenicity of streptococcal antigen I/II polypeptides. Mol Microbiol, 1997; 23: 183–90
14. Nakano K, Tsuji M, Nishimura K et al: Contribution of cell-surface protein antigen PAc of Streptococcus mutans to bacteremia. Microbes Infect, 2006; 8(1): 114–21
15. Monchois V, Willemot RM, Monsan P: Glucansucrases: mechanism of action and structure- function relationships. FEMS Microbiology Reviews, 1999; 23(2): 131–51
16. Long L, Edwards J: Detailed structure of a dextran from a cariogenic bacterium. Carbohydrate Res, 1972; 24: 216–17

17. Matsumura M, Izumi T, Matsumoto M et al: The role of Glucan-binding proteins in the cariogenicity of *Streptococcus mutans*: Microbiol Immunol, 2003; 7(3): 213–15
18. Birkhed D, Rosell KG, Granath K: Structure of extracellular water-soluble polysaccharides synthesized from sucrose by oral strains of *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis* and *Actinomyces viscosus*. Arch Oral Biol, 1979; 24: 53–61
19. Burne RA, Chen YY, Wexler DL et al: Cariogenicity of S.mutans strains with defects in fructan metabolism assessed in a program-fed specific pathogen-free rat model. J Dent Res, 1996; 75: 1572–77
20. Rozen R, Bachrach G, Bronshtetyn M et al: The role of fructans on dental biofilm by *Streptococcus sobrinus*, *Streptococcus mutans*, *Streptococcus gordonii* and *Actinomyces viscosus*. FEMS Microbial Lett, 2001; 195(2); 205–10
21. Loo CY, Mitrakul K, Voss IB et al: Involvement of an inducible fructose phosphotransferase operon in *Streptococcus gordonii* biofilm formation. J Bacteriol, 2003; 185: 6241–54
22. Davey ME, O'Toole GA: Microbial biofilms from ecology to molecular genetics. Microbiol Mol Biol Rev, 2000; 64: 847–67
23. Jefferson KM: What drives bacteria to form a biofilm? FEMS Microbiology Letters, 2004; 236: 163–73
24. Bowden GHW, Hamilton IR: Survival of Oral bacteria. Crit Rev Oral Biol Med, 1998; 9: 54–85
25. Svensater G, Welin J, Wilkins JC et al: Protein expression by planktonic and biofilm cells of *S. mutans*. FEMS Microbiology Letters, 2001; 205: 139–46
26. Drucker DB, Melville JH: Fermentation end products of cariogenic and noncariogenic *Streptococci*. Arch Oral Biol, 1968; 13: 565–70
27. Tanzer JM, Krichevsky MI, Keyes PH: The metabolic fate of glucose catabolized by a washed stationary phase caries-conducive *Streptococcus*. Caries Res, 1969; 3: 167–77
28. Kleinberg I: A mixed-bacteria ecological approach to understanding the role of oral bacteria in dental caries: an alternative to *Streptococcus mutans* and the specific plaque hypothesis. Crit Rev Oral Biol Med, 2002; 13(2): 108–25
29. Nascimento MM, Lemos JAC, Abranches J et al: Adaptive acid tolerance response of *Streptococcus sobrinus*. J. Bacteriol, 2004; 186(19): 6383–90
30. Bender GR, Sutton SCV, Marquis RE: Acid tolerance, proton permeabilities and membrane ATPase of oral *streptococci*. Infect Immun, 1986; 53: 331–38
31. Svensater G, Sjogreen B, Hamilton IR: Multiple stress responses in *S. mutans* and the induction of general and stress-specific proteins. Microbiology, 2000; 146: 107–17
32. van Houte J, de Moor C, Jansen H: Synthesis of iodophilic polysaccharide by human oral streptococci. Arch Oral Biol, 1970; 15: 263–66
33. Freedman ML, Coykendall AL: Variation in intracellular polysaccharide synthesis among *Streptococcus mutans* strain. Infect Immun, 1975; 12: 475–79
34. Harris GS, Michalek SM, Curtiss R III: Cloning of a locus involved in *Streptococcus mutans* intracellular polysaccharide accumulation and virulence testing of an intracellular polysaccharide-deficient mutant. Infect Immun, 1992; 60(8): 3175–85
35. Hamada S, Ooshima T: Production and properties of bacteriocins (mutacins) from *Streptococcus mutans*. Arch Oral Biol, 1975; 20: 641–48
36. Rogers AH: Bacteriocinogeny and properties of some bacteriocins of *Streptococcus mutans*. Arch Oral Biol, 1976; 21: 99–104
37. Hillman JD, Dzuback AL, Andrews SW: Colonization of the human oral cavity by a *Streptococcus mutans* mutant producing increased bacteriocin. J Dent Res, 1987; 66: 1092–94
38. van der Hoeven JS, Rogers AH: Stability of the resident microflora and the bacteriocinogeny of *Streptococcus mutans* as factors affecting its establishment in pathogen-free rats. Infect Immun, 1979; 23(2): 206–12
39. Novak J, Caufield PW, Miller EJ: Isolation and chemical characterization of a novel lantibiotic mutacin from *Streptococcus mutans*. J Bacteriol, 1994; 176: 1316–20
40. Loesche WJ: Nutrition and dental decay in infants. Am J Clin Nutr, 1985; 41: (Suppl.2), 423–35
41. Peterson PE: Continuous improvement of Oral Health in the 21st century. The approach of the WHO global Oral health programme. The World Oral Health Report, WHO 2003
42. Marthaler M: Changes in the prevalence of dental caries. How much can be attributed to changes in diet? Caries Res, 1990; 24(Suppl.): 3–15
43. Burt BA: Relative consumption of sucrose and other sugars: Has it been a factor in reduced caries experience. Caries Res, 1993; 27(Suppl.): 56–63
44. Naylor MN: Diet and the prevention of dental caries. JR Soc Med, 1986; 79(Suppl.): 11–14
45. Gallus S, Scotti L, Negri E et al: Artificial sweeteners and cancer risk in a network of case-control studies. Ann Oncol, 2007; 18: 40–44
46. Soffritti M, Belpoggi F, Esposti DD et al: First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats. Environ Health Perspect, 2006; 114: 379–85
47. Sowers MR, Burt BA, Sohn W: Carbonated soft drinks and dental caries in the primary dentition. J Dent Res, 2006; 85: 262–66
48. Dreizen S, Brown LR: Xerostomia and dental caries. In Loesche WJ, Stiles HM, O'Brien TC (eds.), Proceedings: microbial aspects of dental caries (a special supplement to Microbiology Abstracts), Vol, 1, Information Retrieval, Inc, Washington, D.C.: 1976; 263–74
49. Lowenthal A: Atypical caries of the narcotics addict. Dent Surv, 1967; 44–47
50. Featherstone JD: The science and practice of treating dental caries J Am Dent Assoc, 2000; 131: 887–99
51. Frazier PD, Little MF, Casciani FS: X-ray diffraction analysis of human enamel containing different amounts of fluoride. Arch Oral Biol, 1967; 12: 35–42
52. Brown WE, Gregory TM, Chow LC: Effects of fluoride on enamel solubility and cariostasis. Caries Res, 1977; 11(Suppl.): 118–41
53. Johnston DW: Current status of professionally applied topical fluorides. Community Dent Oral Epidemiol, 1994; 22: 159–63
54. Featherstone JD: Prevention and reversal of dental caries: role of low level fluorides. Community Dent. Oral Epidemiol, 1999; 27: 31–40
55. De Paola PF, Brudevold F, Aasenden R et al: A pilot study of the relationship between caries experience and surface enamel fluoride in man. Arch Oral Biol, 1975; 859–64
56. Karjalainen S: Eating patterns diet and dental caries. Dent Update, 2007; 34: 295–98, 300
57. El-Moung T, Rogers DT, Furr JR et al: Antiseptic induced changes in cell surface of a chlorhexidine sensitive and chlorhexidine resistant strain of *Providencia stuartii*. J Antimicrobial Chemotherapy, 1985; 16: 685–89
58. Mc Donnell G, Russell AD: Antiseptics and Disinfectants: Activity action and resistance. Microbiol Rev, 1999; 12: 147–79
59. Emilson CG: Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res, 1994; 73: 682–91
60. Twetman S: Antimicrobials in future caries control? Caries Res, 2004; 38: 223–29
61. McClure FF, Hewitt WL: The relation of penicillin to induced rat dental caries and oral L. acidophilus. J Dent Res, 1946; 25: 441–43
62. Fitzgerald RJ: Inhibition of experimental dental caries by antibiotics. Antimicrob Agents Chemother, 1972; 1: 296–302
63. Fitzgerald RJ: The potential of antibiotics as caries-control agent. J Am Dent Assoc, 1973; 87: 1006–9
64. Little WA, Thomson LA, Bowen WH: Antibiotic susceptibility of *S. mutans*: comparison of serotype profiles. Antimicrob Agents Chemother, 1979; 15: 440–43
65. Nguyen PTM, Baldeck JD, Olsson J, Marquis RE: Antimicrobial actions of benzimidazoles against oral *Streptococci*. Oral Microbiology Immunology, 2005; 20: 93–100
66. Sulaiman MI, Ajabnoor MA, Al-Khateeb T: Effects of *Salvadora persica* extracts on mice exploratory locomotion activities. J Ethnopharmacology, 1968; 17: 263–68
67. Babay V, Almas K: Effect of miswak extract on healthy human dentin: an *in vitro* study. Saudi Dent J, 1999; 11: 46–52
68. Almas K, Al-Bagieh NH: The antimicrobial effects of bark and pulp extracts of miswak, *Salvadora persica*. Biomed Letters, 1999; 60: 71–75
69. Almas K: The antimicrobial effects of extracts of *Azadiracta indica* (Neem) and *Salvadora persica* chewing sticks. Indian J Dent Res, 1999; 10: 23–26
70. Pai MR, Acharya LD, Udupa N: Evaluation of antiplaque activity of *Azadiracta indica* leaf extract gel – a 6 week clinical study. J Ethnopharmacology, 2004; 90: 99–103
71. Arias ME, Gorney JD, Cadmani NM et al: Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill. Ex Hook et. Am. Life Sci, 2004; 75(2): 191–202
72. Wolinsky LE, Sote EO: Isolation of natural plaque inhibitory substance from Nigerian chewing stick. Caries research, 1984; 18(3): 216–25

73. Rukayadi Y, Hwang JK: *In-vitro* activity of xanthorrhizol against *S. mutans* biofilms. Letters in Applied Microbiology, 2006; 42: 400–4
74. Song JH, Yang TC, Chang KW et al: *In vitro* effects of a fraction separated from *Polygonum cuspidatum* roots on the viability, in suspension and biofilms and biofilm formation of mutans streptococci. J Ethnopharmacol, 2007; 112(3): 419–25
75. Xiao J, Zuo YL, Liu Y, Li JY, Hao YQ and Zhou XD: Effects of *Nidus vespae* extract and chemical fractions on glucosyltransferases, adherence and biofilm formation of *Streptococcus mutans*. Arch Oral Biol, 2007; 52 (9): 869-875
76. Yu HH, Seo SJ, Hur JM et al: *Asarum sieboldii* extracts attenuate growth, acid production, adhesion and water-insoluble glucan synthesis of *Streptococcus mutans*. J Med Food, 2006; 9(4): 505–9
77. Limsong J, Benjavongkulchai E, Kuvatanasuchati J: Inhibitory effect of some herbal extracts on adherence of *S. mutans*. J Ethanopharmacol, 2004; 92: 281–89
78. Prabu GR, Gnanamani A, Sadulla S: Guaijaverin – a plant flavonoid as potential antiplaque agent against *S. mutans*. J of Applied Microbiology, 2006; 101: 487–95
79. Matsumoto M, Minami T, Sasaki H et al: Inhibitory effects of Oolong tea extracts on caries-inducing properties of mutans *Streptococci*. Caries Res, 1999; 33: 441–45
80. Filosche SK, Soma K, Sissons CH: Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Microbiology and Immunology, 2005; 20: 221–25
81. Koo H, Rosalen PL, Cury JA et al: Effect of a new variety of *Apis mellifera* propolis on mutans *Streptococci*. Curr Microbiol, 1999; 41: 192–96
82. Ikeno K, Ikeno T, Miyazawa C: Effect of propolis on dental caries in rats. Caries Res, 1991; 25: 347–51
83. Koo H, Rosalen PL, Cury JA et al: Effect of compounds found in propolis on *S. mutans* growth and on GTF activity. Antimicrob Agents Chemother, 2002; 46: 1302–9
84. Guo JH, Jia R, Fan MW et al: Construction and Immunogenic characterization of a fusion anti-caries DNA vaccine against PAc and glucosyltransferase I of *Streptococcus mutans*. J Dent Res, 2004; 83: 266–70
85. Ajdic D, McShan MW, McLaughlin RL et al: Genome sequence of *S. mutans* UA159, a cariogenic pathogen. Proc Natl Acad Sci USA, 2002; 99: 14434–39
86. Russell MW, Hajishengallis G, Childers NK, Childers SM: Secretory immunity in defense against cariogenic mutans streptococci. Caries Res, 1999; 33: 4–15
87. Smith DJ: Caries Vaccine for the twenty-first century. J Dental Education, 2003; 67: 1130–39
88. Jespersgaard C, Hajishengallis G, Huang Y et al: Protective immunity against *Streptococcus mutans* infection in mice after intranasal immunization with the glucan-binding region of *S. mutans* glucosyltransferase. Infect Immun, 1999; 67: 6543–49
89. Childers NK, Tong G, Michalek SM: Nasal immunization of humans with dehydrated liposomes containing *Streptococcus mutans* antigens by mucosal routes. Oral Microbiol Immunol, 1997; 12: 329–35
90. Childers NK, Tong G, Li F et al: Humans immunized with *Streptococcus mutans* antigens by mucosal routes. J Dent Res, 2002; 81: 48–52
91. Katz J, Harmon CC, Buckner GP et al: Protective salivary immunoglobulin A responses against *Streptococcus mutans* infection after intra nasal immunization with *S. mutans* Antigen I/II coupled to the B subunit of cholera toxin. Infect Immun, 1993; 61: 1964–71
92. Zhang P, Jespersgaard C, Lamberty-Mallory L et al: Enhanced immunogenicity of a genetic chimeric protein consisting of two virulence antigens of *Streptococcus mutans* and protection against infection. Infect Immun, 2002; 70: 6779–87
93. Fan MW, Bian Z, Peng ZX et al: A DNA vaccine encoding a cell-surface protein antigen of *Streptococcus mutans* protects rats from caries. J Dent Res, 2002; 81: 784–87
94. Ferretti JJ, Shea C, Humphrey MW: Cross reactivity of *S. mutans* antigens and human heart tissue. Infect Immun, 1980; 30: 69–73
95. Loimaranta V, Carlen A, Olsson J et al: Concentrated bovine colostral whey proteins from *Streptococcus mutans/Streptococcus sobrinus* immunized cows inhibit the adherence of *Streptococcus mutans* and promote the aggregation of mutans streptococci. J Dairy Res, 1998; 65: 599–607
96. Hamada S, Horikoshi T, Minami T et al: Oral passive immunization against dental caries in rat by use of hen egg yolk antibodies specific for cell-associated glucosyltransferase of *Streptococcus mutans*. Infect Immun, 1991; 51: 4161–67
97. Hatta H, Tsuda K, Ozeki M et al: Passive immunization against dental plaque formation in humans: Effect of a mouth rinse containing egg yolk antibodies (IgY) specific to *Streptococcus mutans*. Caries Res, 1997; 31: 268–74
98. Ma JKC, Hiatt A, Hein M et al: Generation and assembly of secretory antibodies in plants. Science, 1995; 268: 716–19
99. Ma JKC, Hikmat BY, Wykoff K et al: Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med, 1998; 4: 601–6
100. Huang Y, Hajishengallis G, Michalek SM: Induction of protective immunity against *Streptococcus mutans* colonization after mucosal immunization with attenuated *Salmonella enterica* serovar typhimurium expressing an *S.mutans* adhesin under the control of *in vivo*-inducible *nir*B promoter. Infect Immun, 2001; 69: 2154–61
101. Michalek SM, Childers NK, Katz J et al: Liposomes and conjugate vaccines for antigen delivery and induction of mucosal immune responses. Adv Exp Md Biol, 1992; 327: 191–98
102. Smith DJ, Trantolo DJ, King WF et al: Induction of secretory immunity with bioadhesive poly(D, L-lactide-co-glycolide) microparticles containing *Streptococcus sobrinus* glucosyltransferase. Oral Microbiol Immunol, 2000; 15: 124–30
103. Hillman JD, Brooks TA, Michalek SM et al: Construction and characterization of effector strain of *Streptococcus mutans* for replacement therapy of *Streptococcus mutans*. J Dent Res, 2002; 68: 543–49
104. Smith JL, Orugunty RS, Hillman JD: Lantibiotic production by Streptococcus mutans: their uses in replacement therapy for the prevention of dental caries and as antibiotics for the treatment of various infectious diseases. Research and Applications in Bacteriocins, 2007; 95–115
105. Johnson CP, Gross SM, JD Hillman: Cariogenic potential *in vitro* in man and *in vivo* in the rat of LDH mutants of *Streptococcus mutans*. Arch Oral Biol, 1980; 25: 707–13
106. Hillman JD: Genetically modified *Streptococcus mutans* for the prevention of dental caries. Antonie Van Leeuwenhoek, 2002; 82: 361–36
107. 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

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