Present and Future Approaches for the Control of Caries

Kenneth J. Anusavice, Ph.D., D.M.D.

Abstract: This article summarizes current and potential future approaches for the management of caries. Current surveys suggest that traditional “drill, fill, and bill” dentistry is still widely practiced in the United States in spite of considerable evidence that supports a minimally invasive treatment approach. Because there is a wide variability in treatment decisions on when and how to prevent new lesions, on how to arrest the progression of existing lesions, and on when and how to place initial and replacement restorations, the findings from some studies differ significantly from the results of other studies. While fluoride treatments are known to prevent a percentage of new lesions, they do not have the ability to prevent all caries lesions. Modern management of caries entails treating patients according to risk and monitoring early lesions in tooth surfaces that are not cavitated. Although we know that the dmfs score for children is a powerful predictor of caries increment in permanent teeth of these children a few years later, this score is rarely used in private practice as a measure of risk or as a measure of treatment success. Although these modern methods for caries management offer great promise for controlling the disease, they may take decades to apply in a standardized way so that the variability in treatment is reduced. However, during the next two decades, an alternative approach to caries prevention such as replacement therapy and a caries vaccine may become available as a more consistent method of controlling this disease.

Dr. Anusavice is Associate Dean for Research and Chair of the Department of Dental Biomaterials at the University of Florida College of Dentistry. Direct correspondence to him at Department of Dental Biomaterials, University of Florida, College of Dentistry, P.O. Box 100446, Gainesville, FL 32610-0446; 352-392-4351 phone; 352-392-7808 fax; kanusavice@dental.ufl.edu.

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an E1 lesion to be restored. Only twelve of the forty-six states (26 percent) covered by these boards did not allow teeth with E1 or E2 lesions to be surgically treated.

Premature surgical intervention and placement of restorations may lead to overtreatment and the earlier introduction of the restoration life cycle, which may result in larger and larger subsequent replacement restorations and shorter associated survival times. However, as restorations increase in size and cusp replacements are involved in high caries-risk patients, none of the current restorative materials are entirely satisfactory for long-term durability. For restorations with fewer than four surfaces, secondary caries is likely to lead to replacement decisions, while for four-surface situations, fractures are more likely to occur.

Future methods of caries lesion detection and measurements of therapeutic outcomes used to prevent or control the disease will employ to a greater extent improved diagnostic devices that can accurately detect early lesions, “hidden” occlusal lesions, and provide 3D images of the demineralized regions. During the transition period, better diagnostic devices and methods will be introduced to enhance the sensitivity and specificity of caries detection and lesion depth estimation. In addition, some clinicians advocate the use of air abrasion to confirm the suspected presence of carious lesions in pit or fissure areas. It is clear that concepts of minimal intervention dentistry are becoming more widely accepted.

This improved imaging capability will provide greater support to promote the principles of minimally invasive dentistry including caries risk assessment, monitoring of noncavitated carious tooth surfaces, remineralization therapy, and use of “smart” preventive and restorative materials that will improve our ability to monitor disease activity and the outcomes of preservative therapies.

In addition to the use of conventional physical and chemotherapeutic methods of caries management, future prevention methods may also include replacement therapy (probiotics) and/or vaccines. For caries-active individuals, acidogenic and acid-tolerant gram-positive bacteria such as mutans streptococci and lactobacilli abound relative to acid-sensitive species associated with sound enamel. One of the replacement therapy options entails the application of a genetically engineered “effector strain” of S. mutans that will replace the cariogenic or “wild strain” to prevent or arrest caries and to promote optimal remineralization of tooth surfaces that have been demineralized but that have not become cavitated. Another approach is based on a genetic modification of two plaque streptococci to create organisms that produce ammonia from urea and arginine. These organisms will reside in dental plaque, and the ammonia produced from salivary and dietary substrates will prevent the colonization of cariogenic bacteria and ensure internal pH homeostasis.

This review summarizes the principles, benefits, and drawbacks of four caries management approaches likely to coexist in the near future to support our societal need to either prevent this infectious disease or to significantly reduce its activity level and the potential costly consequences of disease progression.

### Physical/Mechanical and Chemotherapeutic Approaches to Caries Management

Caries is a disease caused by a group of oral streptococcal micro-organisms, comprised primarily of S. mutans, that occurs in three phases: 1) initial interaction with the tooth surface mediated by adhesins; 2) accumulation of the bacteria in a biofilm and the production of glucose and glucans by the bacterial enzyme glucosyl transferase; and 3) the formation of lactic acid.

Three current methods of caries management include traditional prevention (prophylaxis and fluoride) and early surgical intervention; traditional prevention and minimal intervention; and risk assessment, prevention assessment, variable recall periods based on risk, lesion monitoring, and delayed intervention. The main steps involved in the third method are shown schematically in Figure 1.

Recent evidence suggests that only 40.9 percent of proximal surfaces of permanent teeth and 28.3 percent of primary teeth with lesions in the outer half of dentin are not cavitated. This means that teeth with such lesions can be monitored rather than restored until the lesion has progressed well into dentin.

Early detection of carious lesions and assessment of disease activity provide a greater opportunity to limit the extent of demineralization associated with the disease process. Bjørndal and Thylstrup demonstrated through histological analyses that the size of a caries lesion along the
dentinoenamel junction (DEJ) is controlled by the size of the outer enamel lesion (Figure 2). The chance to prevent further enamel demineralization and potentially to remineralize the affected enamel structure can markedly limit the size of restorations placed (if needed) and, more importantly, it may eliminate the need for a restoration altogether.

The minimally invasive dentistry approach is based on assessment of caries risk and control of caries as an infectious disease process. Thus, decisions to restore are delayed until it is clear that tooth surfaces are cavitated or are likely to become cavitated despite all practical preventive and remineralization efforts.

According to the study of Pitts and Rimmer, lesions in enamel should not be restored since only 10.5 percent of permanent teeth are likely to be cavitated when proximal lesions are in the inner half of enamel (Figure 3). Thus, a classification of lesion severity is important since decisions on when to restore can be made on a more standardized basis. One common classification system consists of E0 (no lesion), E1 (lesion within the outer half of enamel), E2 (inner half of enamel), D1 (outer third of dentin), D2 (middle third of dentin), and D3 (inner third of dentin). Radiographs of the same tooth with no lesion (E0), an E2 lesion, and a D1 lesion are shown in Figure 4.

The primary public health measures are the use of topical fluoride agents and fluoridated water. Traditional physical/mechanical methods of caries prevention in the United States include oral hygiene procedures (tooth brushing, flossing, and professional tooth debridement). Fluoride-containing and triclosan-containing toothpastes provide chemotherapeutic benefits as well. However, professionally prescribed chemotherapeutic agents are often applied to further reduce the caries risk of susceptible individuals.

Figure 1. Management plan for treatment of caries as an infectious disease

Figure 2. The size of a carious lesion along the DEJ (smaller vertical bar) is related to the size of outer enamel lesion (larger vertical bar).
Chemotherapeutic agents such as those containing fluorine and chlorhexidine may also be prescribed and applied at home, in the office, or in both places. These include fluoride varnish, fluoride gel, chlorhexidine, and fluoride-releasing restoratives. Chlorhexidine varnish is also available in some countries.

Fluoride Toothpaste

Marinho et al.\textsuperscript{30} performed a search of the Cochrane Oral Health Group’s Trials Register (2000) plus several other databases on randomized or quasi-randomized controlled trials with blind outcome assessment to analyze comparative caries prevention data for fluoride toothpaste with placebo in children up to sixteen years during at least one year. The pooled DMFS (decayed, missing, and filled tooth surfaces) prevented fraction was 24 percent (p<0.0001), indicating that 1.6 children need to brush with a fluoride toothpaste (rather than a nonfluoride toothpaste) over three years to prevent one DMFS in populations with an annual caries increment of 2.6 DMFS. In populations with an annual caries increment of 1.1 DMFS, 3.7 children will need to use a fluoride toothpaste for three years to avoid one DMFS. They concluded that the benefits of fluoride toothpaste are firmly established.
However, daily toothbrushing by children with a fluoridated toothpaste alone is unlikely to prevent all new caries lesions. Saporito et al. reported that twice daily toothbrushing with toothpaste containing 0.243 percent NaF or 0.76 percent sodium monofluorophosphate toothpastes by U.S. and Puerto Rican children in the third, fourth, and fifth grades resulted in caries increments (DFS: decayed and filled tooth surfaces) of 1.68 and 1.70, respectively, after one year and 3.56 and 3.56, respectively, after two years.

For adults, the use of a 0.243 percent NaF toothpaste containing 0.3 percent triclosan resulted in a 12.2 percent reduction in caries increment (DFS) after one year and a 16.6 percent reduction after two years. The reduction at two years was significantly greater than that associated with a comparable toothpaste without triclosan. Triclosan (a diphenyl ether [bis-phenyl] derivative, known as either 2,4,4'-trichloro-2'-hydroxydiphenyl ether or 5-Chloro-2-[2,4-dichlorophenoxy] phenol) is a broad-spectrum antibacterial agent with bacteriostatic activity against a wide range of both gram-negative and gram-positive bacteria. Although some studies suggest the safety of triclosan in toothpastes, this topic has been the center of considerable debate, and further investigation is needed to resolve these uncertainties.

Fluoridated Water

The CDC in 2001 issued recommendations regarding the use of fluoride to prevent and control dental caries. Based on studies that suggest frequent exposure to small amounts of daily fluoride will effectively reduce the risk for dental caries in all age groups, the CDC work group recommended that all dentate individuals drink water with an optimal fluoride concentration and brush their teeth twice daily with fluoride toothpaste. However, the group emphasized that additional fluoride measures might be needed for persons at high risk for dental caries and that controlled (measured) use of supplementary fluoride modalities is particularly appropriate during anterior tooth enamel development (age <6 years).

For caries prevention regimens to be as effective as possible, transfer of research evidence to the practicing dental team is crucially important. Based on a survey of 498 U.S. dental hygienists in 2000, Forrest et al. reported that more than 40 percent of the hygienists did not recognize remineralization as the most important mechanism of action of fluoride, and fewer than 50 percent of the survey respondents recognized that dental caries is a chronic infectious disease. An analysis of four factors related to knowledge and practice showed that younger graduates, recent graduates, and members of the American Dental Hygiene Association were more knowledgeable about the effectiveness of caries preventive procedures for children (p<0.01). Although a majority of respondents knew that adults benefited from fluoride and that root caries was an emerging problem, this knowledge was not consistent with treatment provided in their dental practices (p=.02). Less than 35 percent of the hygienists reported that they provide fluoride to adults of any age or that they waited until the disease was present before fluoride is applied. A 1-min application of an APF gel or foam was most often provided to children and adults who were given fluoride treatments. The respondents generally overrated the effectiveness of flossing and toothbrushing while underrating the effectiveness of fluorides.

Fluoride Mouthrinses, Toothpaste, and Gel

Marinho et al. reviewed the literature for randomized or quasi-randomized controlled trials with blind outcome assessment, comparing fluoride mouthrinse with placebo or no treatment in children up to sixteen years during at least one year. The main outcome was caries increment measured by the change in decayed, missing, and filled tooth surfaces (D[M]FS). Based on the results of thirty-four studies, they concluded that fluoride rinses led to a prevented fraction of 26 percent (23 to 30 percent). Thus, in populations of children with a caries increment of 0.25 DFS per year, sixteen children will need to use a mouthrinse to avoid one DFS. In populations having a caries increment of 2.14 DFS, two children would need to rinse to avoid one DFS.

Twetman et al. systematically reviewed and evaluated the scientific literature between 1966 and April 2003 on the caries preventive effect of fluoride toothpastes in various age groups, with special emphasis on fluoride concentration and supervised versus nonsupervised toothbrushing. This systematic search of electronic databases was conducted with the inclusion criteria of a randomized or controlled clinical trial, at least two years follow-up, and caries increment in the permanent dentition (DMFS/T) or primary dentition (DMF/T) as an endpoint. The results of this review suggest 1) strong evidence for
the caries preventive effect of daily use of fluoride toothpaste compared with placebo in the young permanent dentition (prevented fraction of 24.9 percent); 2) a superior preventive effect of toothpastes containing 1,500 ppm F compared with standard toothpastes containing 1,000 ppm F in the young permanent dentition (prevented fraction of 9.7 percent); and 3) higher caries reductions in studies with supervised toothbrushing compared with nonsupervised (prevented fraction of 23.3 percent). Evidence to support the effect of fluoride toothpaste in the primary dentition was incomplete. This study supported the effectiveness of daily toothbrushing with fluoridated toothpastes for caries prevention, although long-term studies are still lacking for adults.

Based on a systematic review of seventy-four studies by Marinho et al., the pooled prevented fraction was 24 percent (21-28 percent). This indicates that 1.6 children would need to brush their teeth with a fluoridated toothpaste to prevent one DFS in populations with a caries increment of 2.6 DFS per year. If the population caries increment was 1.1 D(M)FS per year, 3.7 children would need to use a fluoride toothpaste to avoid one D(M)FS.

Marinho et al. performed a systematic review of twenty-three randomized or quasi-randomized controlled trials with blind outcome assessment, comparing fluoride gel with placebo or no treatment in children up to sixteen years during at least one year. The main outcome was caries increment measured by the change in decayed, missing, and filled tooth surfaces (D[M]FS). They found a prevented fraction of 28 percent (19-37 percent). The prevented fraction (PF) was, on average, 19 percent higher than that in the nonplacebo controlled trials. Based on a comparison with fourteen placebo-controlled trials, a reduction of 21 percent should occur in D(M)FS, indicating that two individuals would have to be treated in a population with a caries increment of 2.2 D(M)FS per year or twenty-four would need to be treated in a population with a caries increment of 0.2 D(M)FS per year.

**Professionally Applied Fluoride Supplemented with Fluoride from Toothpaste**

Splieth et al. raised the question of whether traditional caries prevention programs that target specific groups are still effective. Given the caries decline of approximately 80 percent in children residing in Western Europe and other industrialized countries, they emphasized the need to identify the optimal method to control future caries prevention. Regular fluoride application in a dental office plus the use of a fluoride toothpaste has achieved significant caries reductions over the past two decades. However, these therapies have caused a skewed distribution of high-risk individuals. These investigators suggest that topical fluoride applications at a frequency of six or more times per year combined with effective plaque removal can successfully prevent caries in high caries-risk groups. They further conclude that oral health promotion programs that are only educational in nature and do not include fluoride treatment may not be effective. Furthermore, preventive measures performed at home or in a private practice are associated with minimal compliance in high-risk groups. Thus, they suggest that outreach programs that ensure more consistent control over caries management will be more effective.

Marinho et al. concluded that topical fluorides (mouthrinses, gels, or varnishes) used in addition to fluoride toothpaste achieved a modest caries reduction (10 percent prevented fraction, p=0.01) compared to toothpaste alone. The combined use of fluoride gel and a fluoride mouthrinse resulted in a prevented fraction of 23 percent (p=0.02).

Zimmer et al. conducted a randomized controlled clinical trial on high-risk children who received professional tooth cleaning and an application of 0.1 percent NaF fluoride varnish four times per year and concluded that “it might not be possible to prevent caries in high-risk children by means of the described program.”

**Fluoride Varnish**

Based on another systematic review, Bader et al. assessed the strength of the evidence for the efficacy of professional caries preventive methods for high caries-risk individuals and the efficacy of professional treatment regimens to arrest or reverse noncavitated carious lesions. A search of 1,435 articles resulted in twenty-two studies that evaluated the prevention of carious lesions in caries-active or high-risk individuals. Overall, the strength of the evidence was “fair” for fluoride varnishes and “insufficient” for all other methods. For seven other studies related to the management of noncavitated carious lesions, the strength of evidence for efficacy was “insufficient” for all treatment methods. These results suggest that our previous data on the efficacy
of the methods are inadequate to permit definitive recommendations to be made for individual patients with specific caries risk levels.

Mejare et al.\textsuperscript{44} also performed a systematic review and reported that resin sealants produced a relative caries reduction of 33 percent over a period of at least two years in permanent first molars of children up to age fourteen. They concluded that there is limited evidence to prove that fissure sealing of first permanent molars with resin-based materials has a caries-preventive effect and that the evidence is incomplete for permanent second molars, premolars, and primary molars and for glass ionomer cements used as sealants.

A Cochrane Review of sealants in the permanent teeth of five to ten year old children by Ahovuo-Saloranta et al.\textsuperscript{45} revealed caries reductions ranging from 86 percent at twelve months to 57 percent at forty-eight to fifty-four months. The authors recommended sealing occlusal surfaces of permanent molars with resin-based sealants to prevent caries although they recommend that the caries prevalence level of both individuals and the population should be taken into account.

Petersson et al.\textsuperscript{46} evaluated the caries-preventive effect of professional fluoride varnish treatments based on a systematic search of the literature for articles published between 1966 and August 2003. Of 302 identified papers, twenty-four reports were included from randomized and controlled clinical trials comparing fluoride varnish with placebo, no active treatment, or other fluoride preventive regimens with at least two years' duration. The results suggest limited evidence for the caries preventive effect of topical fluoride varnish applied to permanent teeth. The average prevented fraction was 30 percent (0-69 percent) compared with untreated controls. Inconclusive evidence was reported for fluoride varnish application to primary teeth and to posterior adult teeth.

Marinho et al.\textsuperscript{47} conducted an evidence-based review of randomized or quasi-randomized controlled trials with blind outcome assessment for the use of fluoride varnish in children up to sixteen years during at least one year. They reported a prevented fraction (DFS) of 46 percent (30 to 63 percent) based on seven selected studies. This review suggests a substantial caries-inhibiting effect of fluoride varnish in both the permanent and the deciduous dentitions based largely on trials with no treatment controls.

**Antibacterial and Antimicrobial and Bactericidal Agents**

Twetman\textsuperscript{48} examined recent evidence on the use of antibacterial agents to prevent and control caries and concluded that there is limited evidence for the effectiveness of chlorhexidine (CHX) gels, rinses, and toothpaste in preventing caries in the permanent teeth of children and adolescents. Twenty-two of the interventions in controlled clinical trials from 1995 to May 2003 were related to CHX-containing varnishes. According to the ranking system of the Swedish Council on Technology Assessment in Health Care, the evidence for an anticaries effect of CHX varnishes was inconclusive for caries-active schoolchildren and adolescents with regular fluoride exposure. A preventive effect of CHX varnishes on fissure caries was demonstrated in four out of five studies, when compared with no treatment in children with low fluoride exposure. The evidence for arresting root caries in dry-mouth patients and frail elderly subjects was also inconclusive.

Van Rijkom et al.\textsuperscript{49} performed a meta analysis and reported a 46 percent prevented caries fraction for individuals treated with chlorhexidine. Multiple regression analysis revealed no significant difference among the prevented fractions as a function of application method, application frequency, caries risk, fluoride regimen, and tooth surface. The prevented fraction of chlorhexidine (46 percent) appears to be comparable to that associated with the use of fluoride varnish (Figure 5), and it tends to be more effective than fluoride gels, rinses, and fluoride-containing toothpaste.

One of the most controversial issues regarding caries prevention using bactericidal or antibacterial agents is whether chlorhexidine can be combined with fluoride in either a gel or solution form. Some evidence suggests that the positively charged chlorhexidine ion and the negatively charged fluoride ion do not necessarily negate the action of each other. Katz\textsuperscript{50} reported that individuals who were irradiated for head and neck cancer and who received a combined treatment of four topical applications of 1.0 percent sodium fluoride-1.0 percent chlorhexidine digluconate gel plus daily rinses with an 0.05 percent sodium fluoride-0.2 percent chlorhexidine solution had no new lesions in the subsequent six- to ten-month period. This treatment also resulted in remineralization of existing incipient lesions. The chlorhexidine-fluoride rinses alone also prevented
radiation caries but did not permit remineralization to occur. The four topical applications with a fluoride gel and daily rinses with an 0.05 percent sodium fluoride solution were inadequate to prevent radiation caries.

Ogaard et al.51 conducted a randomized prospective clinical study with 220 patients scheduled for fixed orthodontic therapy to test the hypothesis that application of Cervitec: antimicrobial varnish, which contained 1 percent chlorhexidine plus 1 percent thymol (Ivoclar Vivadent, Schaan, Liechtenstein) in combination with Fluor Protector (Ivoclar Vivadent, Schaan, Liechtenstein), a varnish containing 5 percent difluorosilane (Group 1) was significantly more effective in reducing white spot lesions on the facial surfaces than application of the fluoride varnish alone (Group 2). The antimicrobial varnish significantly reduced the number of mutans streptococci in plaque during the first forty-eight weeks of treatment. This result was not associated with significantly fewer white spot lesions on the facial surfaces compared with the group receiving only the fluoride varnish application. However, the combination of the antimicrobial and fluoride varnishes more effectively reduced the caries increment for the maxillary incisors. The investigators speculated that this was partly caused by an inhibiting effect of the antimicrobial varnish in an area with low oral clearance of fluorine ions and partly by an inhibiting effect of the varnish on mutans streptococci (ms).

Tenovuo et al.52 demonstrated that if mothers with ms levels higher than 10⁵ CFU/mL were given 1 percent chlorhexidine-0.2 percent sodium fluoride gel treatments twice a year for three years (Group 1), the primary teeth of their children (from age one to four years) would have less colonization by ms and they would have fewer lesions than the children of mothers with high ms counts (>10⁵ CFU/mL) who did not receive the combined gel treatment (Control Group 2). In the total study population of 151 children, 16 percent, 42 percent, and 54 percent of the children were colonized by ms by the ages of two, three, and four years, respectively. Most children were colonized only by S. mutans, but two had both S. mutans and S. sobrinus, and two had only S.

Figure 5. Prevented fraction (ΔDMFS) for various prevention methods

FG=fluoride gel; TP=fluoride-containing toothpaste; FR=fluoride rinse; V1=fluoride varnish for primary teeth; V=all fluoride varnish applications; V2=fluoride varnish for permanent teeth; CHX=all chlorhexidine applications; SEAL=pit and fissure sealant
sobrinus. Twenty-eight percent of the ms-positive children developed caries by the age of four years, whereas 14.8 percent of the children with dental caries did not have any detectable ms in their plaque samples. The colonization by ms and the caries incidence were highest in the children of Control Group 1 and lower in the children of mothers in experimental Group 1 and in Control Group 2 (ms counts of <10^5 CFU/mL and no gel treatments). The results of this study suggest that the reduction of maternal salivary ms at the time of tooth emergence may delay, or even prevent, the colonization of ms in the children’s primary teeth with a corresponding decrease in caries incidence, even in a population with an already low prevalence of dental caries.

Ullsfoss et al.53 investigated the effect of two daily rinses with 2.2 mM chlorhexidine and one daily rinse with 11.9 mM NaF in an in vivo human caries model using plaque-retaining bands on premolar teeth scheduled for extraction. A total of twenty-eight teeth were fitted with the bands for four weeks. The tooth surfaces were analyzed by microradiography after the teeth were extracted. The combination of chlorhexidine and fluoride rinses resulted in a slightly greater loss of enamel mineral compared with that observed in “sound” enamel and clearly less than that associated with fluoride rinses alone. Both total plaque bacteria and S. mutans were reduced by chlorhexidine rinses.

**Xylitol**

There appears to be some benefit for caries prevention by using xylitol as a sugar substitute in toothpaste, chewing gum, and other products used or consumed intraorally. Mäkinen et al.54 reported a significant reduction in caries increment for children who were given a xylitol-containing chewing gum for up to five times per day. Isokangas et al.55 have shown that regular maternal use of xylitol chewing gum by 195 mothers with high salivary ms levels resulted in a statistically significant reduction in ms colonization in their children’s teeth at the age of two compared with the teeth of children whose mothers received fluoride or chlorhexidine varnish treatment. At the age of five, the dentinal caries (DMFS) in the xylitol group was reduced by about 70 percent compared with that in the fluoride or chlorhexidine groups. The authors concluded that the maternal use of xylitol chewing gum can prevent dental caries in children by preventing the transmission of mutans streptococci from mother to child. However, based on a systematic review of eighteen articles that met the criteria suggested by the Swedish Council on Technology in Health Care, Lingström et al.56 concluded that the evidence for the use of sorbitol or xylitol in chewing gum was inconclusive and they recommended more well-designed, randomized clinical trials with adequate control groups and acceptable compliance.

**Ozone Technology**

The ability of ozone gas (O3) to kill bacteria, fungi, and viruses is well known.57,58 However, although useful bactericidal action against a variety of human pathogens has been reported for ozone concentrations between 0.3 to 0.9 ppm, these bactericidal ozone concentrations are close to the limit permitted for human exposure.59 Few well-controlled studies have been performed to investigate the bactericidal effect of various doses of ozone gas on oral microorganisms. Oizumi et al.60 reported that an ozone generator using 20 mg/h of ozone was required to disinfect dentures that contained Streptococcus mutans (strain IID 973), Staphylococcus aureus (strain 209-P), and Candida albicans (strain LAM 14322).

The evidence to support the use of ozone gas to prevent caries and to enhance remineralization of demineralized enamel is limited. In vitro and in vivo reports support the potential to arrest caries and to possibly remineralize demineralized tooth structure.60-62 Rickard et al.63 performed a systematic assessment of the scientific literature to assess whether ozone is effective in arresting or reversing the progression of dental caries. Three trials were included, with a combined total of 432 randomized lesions (137 participants). Individual studies revealed inconsistent effects of ozone on management of caries lesions as a function of different measures of caries progression or regression. Few studies of secondary outcomes have been performed, and only one trial has reported an absence of adverse events. Because of the high risk of bias in the available studies and the lack of consistent results between different outcome measures, the authors conclude that there is no reliable evidence to support the application of ozone gas to the surface of decayed teeth to arrest or reverse the demineralization process. They concluded that more evidence of appropriate rigor and quality is required before ozone can be accepted for primary dental care or as an alternative to current methods for the management and treatment of dental caries.
Summary of Current Treatment Methods

Clearly, systematic reviews of the literature do not produce conclusive support on the best methods for preventing new caries lesions or preventing the propagation of existing lesions. There is even far less evidence to support the use of any therapeutic regimens to prevent or control secondary caries although some evidence exists to support the assumption that secondary caries behaves like primary caries.64,65 However, we must apply the “best knowledge” available to control or prevent the disease in this population of individuals at risk for the disease. In the absence of a comprehensive body of conclusive clinical results, modern caries management must be based on the principles of disease management. These include accurate lesion detection, classification of lesion severity, assessment of caries risk, matching of treatment to the risk level, monitoring for evidence of remineralization or further demineralization, and assigning recall intervals according to treatment outcomes and current risk levels.

A remaining concern regarding the multiple use of fluoride regimens is the risk for fluorosis. Because of limited data on the cumulative ingestion of fluoride from drinking water, fluoride-containing toothpaste, and other sources, the prevalence of fluorosis should be monitored to ensure that children are not being overdosed with fluoride used for caries treatment.66

Replacement Therapy

Among indigenous oral microorganisms are those bacteria that have a significant pathogenic potential for the host. Caries-promoting bacteria include \textit{S. mutans} and lactobacilli. Although \textit{S. mutans} has an affinity for attachment to tooth surfaces, \textit{Lactobacillus casei} and \textit{Lactobacillus fermenti} have a low affinity for oral surfaces, suggesting that their association with carious lesions may be related to mechanical adherence.67 Scientists have investigated numerous mechanisms to intervene in these bacterial interactions. Ingestion of probiotic bacteria, particularly lactobacilli,68 is commonly practiced to promote well-balanced intestinal microflora. As bacterial resistance to antimicrobial agents has increased, so too has research into colonization of human tissues with specific effector strains capable of competing against known bacterial pathogens. Recent progress is particularly evident in the application of avirulent \textit{Streptococcus mutans} to control dental caries, alpha hemolytic streptococci to reduce otitis media recurrences, and \textit{Streptococcus salivarius} to prevent streptococcal pharyngitis.69

Replacement therapy involves the use of a harmless effector strain that is permanently colonized in the host’s microflora. This effector strain is designed to prevent the colonization or outgrowth of a particular pathogen.

Many reports have described both positive and negative bacterial interactions in which a specific indigenous microorganism promotes or blocks the presence of a pathogen. To prevent an infection using replacement therapy (recently referred to as probiotic therapy), a natural or genetically modified effector strain is used to intentionally colonize the sites in susceptible host tissues that are normally colonized by a pathogen. If the effector strain is better adapted than the pathogen, colonization or outgrowth of the pathogen will be prevented by blocking the attachment sites, by competing for essential nutrients, or via other mechanisms. As long as the effector strain persists as a resident of the indigenous flora, the host is protected potentially for an unlimited period of time.

\textit{S. mutans} strain BCS3-L1 is a genetically modified effector strain designed for use in replacement therapy to prevent dental caries.23 To be an effective effector strain, BCS3-L1 must satisfy four prerequisites:

1. It must have a significantly reduced pathogenic potential to promote caries.70
2. It must persistently colonize the \textit{S. mutans} sites, thereby preventing colonization by disease-causing strains whenever the host comes into contact with them.
3. It must aggressively displace indigenous strains of \textit{S. mutans} and allow previously infected subjects to be treated with replacement therapy.
4. It must be safe and not make the host susceptible to other disease conditions.

Regarding its pathogenicity, lactate dehydrogenase (LDH) deficiency can be used as the approach for reducing acidogenicity in the construction of the BCS3-L1 strain.70 Cloning the structural gene encoding the \textit{S. mutans} LDH provided the basis for producing LDH-deficient clones,71,72 suggesting that LDH-deficiency was a lethal mutation in most \textit{S. mutans} strains. However, at high sugar concentrations, the levels of activity of these enzymes are ap-
parently insufficient to compensate for the absence of LDH. A supplemental alcohol dehydrogenase (ADH) activity can complement the LDH deficiency when expressed in the temperature sensitive LDH mutant.73

Recombinant DNA technology was used to delete the gene encoding lactate dehydrogenase in BCS3-L1 making it unable to produce lactic acid.24 This effector strain was also designed to produce elevated amounts of a novel peptide antibiotic called mutacin 1140 that gives it a strong selective advantage over most other strains of S. mutans. This effector strain has shown no measurable LDH activity, and it induces a tenfold elevated level of ADH activity relative to its JH1140 parent. Fermentation end-product analysis revealed that BCS3-L1 made no detectable lactic acid.23 As predicted from earlier work,74 most of the metabolized carbon was converted to the neutral end-products, ethanol and acetoin.

Under various cultivation conditions, including growth on a variety of sugars and polyols, such as sucrose, fructose, lactose, mannitol, and sorbitol, BCS3-L1 yielded final pH values that were 0.4 to 1.2 pH units higher than those of its parent, JH1140. The reduced acidogenic potential of BCS3-L1 resulted in a greatly decreased cariogenic potential as shown in several animal models.25 BCS3-L1 was significantly less cariogenic than JH1140 in both gnotobiotic- and conventional-rodent models. It colonized the teeth of conventional rats as well as JH1140 in both aggressive-displacement and preemptive-colonization models. No gross or microscopic abnormalities of major organs were associated with oral colonization of rats with BCS3-L1 for a period of six months.23 The results of these studies provided strong evidence that an LDH-deficient S. mutans strain such as BCS3-L1 has significantly reduced pathogenic potential, and thus satisfies the first prerequisite for use as an effector strain in replacement therapy for dental caries.

Transmission of mutans streptococci within the human population has been extensively studied. Most studies support the idea that this organism is usually transmitted from mother (primary caretaker) to child within a several year period following the onset of tooth eruption.75-79 Other studies75-83 have demonstrated the difficulty of maintaining laboratory strains of mutans streptococci in the mouths of humans, especially when they already had an indigenous strain of this organism.

From a standpoint of replacement therapy for caries prevention, implantation of an effector strain would best be achieved in children immediately after tooth eruption and before the acquisition of a caries-inducing strain. To prevent overcolonization by wild-type strains when the host comes in contact with them, an effector strain should have some significant selective advantage to colonization. This would also enable subjects who have already been infected with a caries-inducing strain of S. mutans to be treated by replacement therapy. Mutacin 1140 is capable of killing virtually all other strains of mutans streptococci against which it was tested.84

To serve as an effector strain for the prevention of dental caries, BCS3-L1 must be genetically stable. Sufficient mutacin 1140 has not been purified to directly test its toxicity. However, the prototype lantibiotic, nisin, is known to have extremely low toxicity,85,86 and has been developed and used for decades as a food preservative that is generally recognized as safe.

It is conceivable that mutacin production by BCS3-L1 and the fermentation products resulting from LDH deficiency could alter plaque ecology and produce another microorganism with pathogenic potential. The mutacin 1140 producing strain of S. mutans eliminated mutacin-sensitive indigenous strains of S. mutans but had no effect on indigenous S. oralis strains that were equally sensitive to mutacin killing in vitro.25 These results indicate that S. mutans has a physically distinct habitat that is separated from the S. oralis habitat by a distance sufficient for dilution to reduce the concentration of mutacin below its minimal inhibitory concentration. A similar explanation could account for the failure to observe qualitative or quantitative changes in the plaque of rats following long-term infection with an LDH deficient mutant, even though the mutant’s metabolic end-products are certain to be different from those of the wild-type strain.74

A final aspect of replacement therapy safety is the requirement for controlled spread of the effector strain within the population. Mutacin 1140 up-production clearly provides a selective advantage to BCS3-L1 colonization. However, the minimum infectious dose has not been determined for this strain or any S. mutans strain in humans. Wives and children of the two subjects infected with the mutacin up-producing S. mutans strain were not colonized when tested fourteen years after the initial infection regimen (J.D. Hillman, personal communication). Obviously, further studies with larger populations need to be performed to measure the potential for horizontal transmission. It is expected that, like wild-
type strains of *S. mutans*, vertical transmission of BCS3-L1 from mother to child will occur at a high frequency. The reduced pathogenic potential of the BCS3-L1 probiotic strain, its proven colonization potential, and its genetic stability support its potential use as an effector strain for replacement therapy to prevent dental caries in human populations at risk for this disease. The main advantages of this replacement therapy include the lifelong protection provided by a single application, the negligible risk for untoward results, and the lack of a need for patient education and compliance that are required for conventional oral hygiene regimens.

A clinical trial began early in 2005 to test the effectiveness of replacement therapy. Thus, it is too early to determine the potential of this treatment method to prevent new caries lesions and to arrest existing lesions without any significant side effects.

**Genetically Engineered, Alkali-Producing Streptococci**

The pH of plaque fluid is a key environmental factor affecting the physiology, ecology, and pathogenicity of the oral biofilms colonizing the hard tissues of the human mouth. Much attention has been focused on controlling organic acids produced through the metabolism of carbohydrates by pathogenic oral bacteria. Oral bacteria can be genetically modified to produce alkali environments, which may be beneficial in preventing or arresting the caries process. Recent evidence suggests that alkali generation may play a major role in pH homeostasis in oral biofilms and it may moderate initiation and progression of dental caries. In a brief review, Burne and Marquis have described a process of alkali generation resulting from ammonia produced from arginine and urea. This process is associated with a genetically altered strain of streptococci interacting with components of dental plaque. No data are yet available from randomized, controlled clinical trials to support the application of this potential therapy.

**Caries Vaccine**

This section describes methods by which mucosal host defenses can be induced by immunization to interfere with the colonization of mutants streptococci. Anticaries vaccines operate on the principle of reducing the population of the indigenous bacteria that are associated with the caries disease process. The two-step process of vaccine development involves identification of specific antigens of mutants streptococci against which protective immune responses can be induced, and the application of an immunization treatment method that will sustain adequate levels of salivary antibodies. Key antigens include streptococcal surface proteins that control attachment to tooth surfaces and glucosyltransferases that produce adhesive glucans from sucrose. Oral application of specific antibodies against selected antigens of mutants streptococci (passive immunization) has produced promising results.

The feasibility of immunizing experimental animals with protein antigens obtained from *Streptococcus mutans* against oral colonization by mutants streptococci has been demonstrated in several studies. Immunization is induced by IgA antibodies that can inhibit mechanisms of streptococcal accumulation on tooth surfaces depending on the choice of vaccine antigen. Mucosal immunization is designed to induce high levels of salivary antibodies that can be sustained for extended periods and to ensure so-called “immune memory.” Human studies have shown that passively applied salivary antibodies to mutants streptococci can suppress recolonization by mutants streptococci. However, validation of vaccine effectiveness will depend on the performance of candidate vaccines in clinical trials.

Some methods of mucosal vaccine antigen delivery have resulted in inhibition of dental caries associated with *S. mutans* infection. Although passive administration of antibodies to virulence antigens of *S. mutans* has shown some promise, the caries-protective benefits of active immunization using caries vaccines must be proven in pediatric clinical trials.

For a caries vaccine to be accepted by the dental profession, many questions need to be answered. One of the most important questions is: what will be the long-term effect of altering the indigenous oral microflora? Also, can the highest caries activity level of infection caused by the pathogen, *Streptococcus mutans*, be inactivated immunologically? Which entry pathways of *S. mutans* into the dental biofilm can be controlled by immunization? Can an immune response be induced by virulence factors associated with *S. mutans*? How safe are caries vaccines relative to other caries prevention regimens? Will the profession adopt vaccination as a caries prevention mechanism given the greatly reduced caries prevalence over the past several decades?

Considerable evidence exists to confirm *Streptococcus mutans* as the primary caries-inducing microorganism, and a cell-surface protein antigen,
and glucosyltransferases and glucan binding proteins as major colonization factors.\textsuperscript{91-99} It is believed that mucosal induction of salivary IgA antibody to glucosyltransferases inhibits the attachment to and accumulation of \textit{S. mutans} on hard tissue. A nasal spray vaccine produces a better mucosal IgA response compared with oral and tonsilar administration. Human trials should focus initially on Phase I trials on pre-adolescents and later on Phase I, II, and III trials on infants prior to tooth eruption.

Adherence of \textit{S. mutans} bacteria to tooth tissues is a prerequisite for colonization. Other evidence supports the need for vaccines or other therapies to inhibit specific virulence factors to prevent caries. Active and passive immunization processes have been developed for immunotherapy against dental caries. Significant caries inhibition effects have been shown in experimental mice, rats, and monkeys, which have been immunized subcutaneously, orally,\textsuperscript{96} or intranasally\textsuperscript{97} with these antigens. However, only a few studies have examined the efficacy of dental caries vaccines in humans. Recently, local passive immunization using murine monoclonal antibodies, transgenic plant antibodies, egg-yolk antibodies, and bovine milk antibodies to antigens of \textit{mutans} streptococci have been applied to control bacterial colonization and dental caries in humans. Such immunization is believed to be a safer approach for controlling dental caries than active immunization.\textsuperscript{96}

Russell et al.\textsuperscript{96} and Wu and Russell\textsuperscript{100} focused on the saliva-binding region where certain residues appear to be important in attachment to the salivary pellicle on the tooth surface. Antibodies against this part of the molecule can exert an anti-adherence function. Antibodies against antigen I-II are effective anti-adherence antibodies. There is no evidence that antigen I-II has cardiovascular cross reactivity.

Recently, investigators have shifted their focus toward mucosal vaccines that employ the immunogenicity of cholera toxin and its B subunit using rat and monkey models or toward the saliva-binding region in the rat model that is genetically coupled to the nontoxic components of antigen I-II. However, no longitudinal clinical data from infancy onward are available to demonstrate a correlation between antibodies to antigen I-II and a decrease in the concentration of \textit{S. mutans} colonies.\textsuperscript{101} In this regard, many obstacles remain for vaccine development to be successful including the high costs required and the lack of prioritization of caries vaccines in the last Institute of Medicine report. Other uncertainties include the number of contacts that will be required with a primary provider, the overall delivery cost, the benefits versus risks of active and passive immunity approaches, the role of industry, and acceptance by the dental profession and the public.

## Conclusion

Any method of caries management must deal with one or more of the three main stages of the disease process: 1) the initial interaction of bacterial cells with the tooth that is mediated by adhesins; 2) the colonization and growth of cariogenic bacteria in a biofilm; and 3) the production of glucose and glycans by glucosyl transferase, a bacterial enzyme, which affects the production of lactic acid that initiates the demineralization process. Chemical agents are designed to disrupt cell metabolism and to kill all disease-producing cells or a significant percentage of the cells. This therapy is designed either to prevent the disease process or to cause a reduction in disease-related manifestations.

In general, replacement therapy employs a carefully constructed effector strain that provides a number of advantages over conventional prevention strategies and oral vaccines. In the case of dental caries, a single colonization regimen that leads to persistent colonization by the effector strain should provide lifelong protection. In the event that the effector strain does not persist indefinitely in some subjects, reapplication can be performed as the need arises without significant added concern for safety or effectiveness. One of the greatest advantages of replacement therapy and caries vaccination is that there is minimal need for patient compliance relative to caries prevention although oral hygiene measures to prevent periodontal disease will still be required.

The development of a vaccine useful against caries infection faces an even greater challenge because of the elimination of one of the commensal oral microorganisms. Before any vaccine is brought to market, the long-term consequences of disturbing the commensal microflora of the oral cavity that has evolved over many centuries must be determined.

The current resurgence of various infectious diseases indicates that traditional and antibacterial-based therapies alone will not suffice. The continued study of bacterial interactions as they occur \textit{in vivo} will inevitably lead to the identification of naturally occurring effector strains for the replacement therapy of various infections. If ultimately successful, the use of genetic engineering to tailor an effec-
tor strain for replacement therapy of dental caries will encourage similar efforts to prevent other infectious diseases.

However, until such time as these alternative therapies are proven to be safe and effective for humans, conventional caries prevention methods primarily based on applications of fluoride must be used. Of the currently available methods, treatment regimens should be designed according to caries risk levels. Conscientious use of conventional prevention methods (brushing, flossing, topical fluoride, controlled sugar consumption, etc.) is sufficient in most cases to maintain the S. mutans level below its minimal pathogenic concentration. The fact that dental caries remains as one of the most common infectious diseases afflicting humans is a clear indication that a truly effective prevention strategy cannot rely on patient compliance.

For low-risk patients, minimal fluoride exposure is indicated. If fluoride treatment is indicated because of inadequate water fluoride content or the presence of other major risk factors, a low-dose, high-frequency application is recommended. For moderate-risk and high-risk patients, antibacterial or high-frequency application is recommended. For the presence of other major risk factors, a low-dose, because of inadequate water fluoride content or sure is indicated. If fluoride treatment is indicated on patient compliance.

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REFERENCES


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