Ecological Approaches to Dental Caries Prevention: Paradigm Shift or Shibboleth?

Nebu Philip^a Bharat Suneja^b Laurence J. Walsh^a

^aSchool of Dentistry, The University of Queensland, Brisbane, QLD, Australia; ^bBaba Jaswant Singh Dental College and Hospital, Ludhiana, India

Keywords

Biofilm ecology · Dental caries · Prevention

Abstract

Contemporary paradigms of dental caries aetiology focus on the ecology of the dental plaque biofilm and how local environmental factors can modulate this to cause disease. The crucial role that a healthy oral microbiome plays in preventing caries and promoting oral health is also being increasingly recognized. Based on these concepts, several ecological preventive approaches have been developed that could potentially broaden the arsenal of currently available caries-preventive measures. Many of these ecological approaches aim for long-term caries control by either disrupting cariogenic virulence factors without affecting bacterial viability, or include measures that can enhance the growth of health-associated, microbially diverse communities in the oral microbiome. This paper argues for the need to develop ecological preventive measures that go beyond conventional caries-preventive methods, and discusses whether these ecological approaches can be effective in reducing the severity of caries by promoting stable, health-associated oral biofilm communities.

© 2018 S. Karger AG, Basel

Oral health is integral to general well-being, with profound individual and societal implications that extend well beyond the functions of the craniofacial complex. Although largely preventable, diseases such as dental caries are major public health concerns, imposing a costly burden on health services. Traditional caries epidemiological measures do not adequately reflect the social impacts, economic costs, and health care system effects of the disease [Casamassimo et al., 2009].

At the individual level, control of dental caries remains largely dependent on twice-daily mechanical oral hygiene in the form of toothbrushing with fluoride dentifrices, a preventive approach that has been in place for over 50 years. Based on the current understanding of the dental caries process, several ecological preventive strategies have been developed or are currently under investigation, suggesting a future where caries prevention will not be narrowly focussed on fluoride therapies. This review will explore the rationale supporting the need for alternate methods of dental caries control, and will discuss the current status of some ecological approaches to biofilm modification and caries prevention.

Dental Caries: A Biofilm-Mediated Multifactorial Disease

Dental caries belongs to a group of diseases that are considered “complex” or “multifactorial,” with no single causation pathway, and therefore, are not amenable to simplistic preventive solutions such as the elimination of “one type of organism” or merely enhancing “tooth resistance” [Fejerskov, 2004]. Dental caries is now widely recognized to be an endogenous, biofilm-mediated disease that occurs when acidogenic/aciduric members of resident oral flora obtain a selective ecological advantage over other species, disrupting the homeostatic balance of the plaque biofilm and initiating the disease process [Marsh and Martin, 1999]. Modern molecular analyses and microbial culture techniques have demonstrated that an entire range of bacteria, not just mutans streptococci (MS) or lactobacilli (LB), can contribute to the caries process at different stages [Tanner et al., 2016], and that even fungi such as *Candida albicans* can significantly enhance the cariogenic virulence of plaque biofilms [Koo and Bowen, 2014]. An MS- or LB-dominated microbiome may be found only at the advanced stages of the disease, where the increased severity and frequency of biofilm acidification results in the oral microbiome becoming less microbially diverse [Takahashi and Nyvad, 2008]. Indeed, it is not the bacterial genotype per se but their shared phenotypic characteristics (being acidogenic and aciduric) that are more important for driving the microbial ecological shift that leads to dental caries [Takahashi and Nyvad, 2011].

While the ionic aspects of the dental caries process have been the focus of research for decades, new insights into the aetiology and microbial aspects of this biofilm-mediated disease have engendered novel concepts and approaches for its prevention and control [ten Cate and Cummins, 2013]. A consensus is now emerging that caries-preventive measures should aim to not only correct the environmental pressures responsible for the plaque biofilm dysbiosis, but also help to maintain a healthy, microbially diverse, resident microbiome [Marsh et al., 2015]. These new approaches acknowledge the possibility that bacteria yet to be identified and cultured may participate in the caries process, and aligns with engaging in a more comprehensive approach towards preventing dental caries as a disease at the patient level [Walsh, 2011].

Rationale for Newer Methods of Dental Caries Control

Ecological Strategies

The historical focus in preventing and managing oral diseases was on eliminating dental plaque from teeth. However, contemporary evolving evidence is increasingly highlighting the beneficial aspects of a healthy oral microbiome [Kilian et al., 2016]. Commensal plaque microflora have a symbiotic relationship with the host, not only acting as a barrier to opportunistic pathogens, but also carrying out metabolic processes that benefit the host [He et al., 2011; Schlafer et al., 2017]. Acute infections of the oral mucosa are rare because of the interplay between the host immune system and microbial symbionts [Zaura et al., 2014]. Likewise, the pro- and anti-inflammatory activities of resident bacteria help maintain homeostasis in the oral cavity [Devine et al., 2015]. A moderate amount of healthy plaque has also been shown to prevent erosive enamel lesions and hypersensitivity [Honorio et al., 2010].

Based on this changed understanding of the importance of a healthy oral microbiome, caries-preventive strategies should ideally take an ecological approach to the holobiont. Indiscriminate or “shotgun” suppression of almost the entire oral biota, without understanding the overall effects on plaque ecology, is unlikely to have long-term success in controlling the disease [Caufield et al., 2001]. Instead, preventive and curative products should either specifically target cariogenic bacteria without affecting other resident microflora, or they should inhibit virulence factors (e.g., glucan synthesis or acid production) rather than bacterial viability [ten Cate and Zaura, 2012]. Measures that enhance colonization of health-promoting microbial communities can also help in correcting the ecological imbalance in cariogenic biofilms. The advantage of such an approach is that the impacts of low pH environments generated by acidogenic organisms can be counterbalanced by ammonia production from other bacteria. Following an ecological approach to caries prevention can potentially preserve the favourable effects that the host derives from the resident oral microbiome, while reducing cariogenic virulence factors that are responsible for plaque biofilm dysbiosis.

Aetiological Factors

The control and prevention of any disease should preferably focus on the aetiological factors involved. For dental caries, this would be the periodic disorganization of the oral plaque biofilm by mechanical oral hygiene, along

with dietary modification to reduce exposure to fermentable carbohydrates [Cury and Tenuta, 2008]. Unfortunately, individual oral hygiene measures have only a limited impact in caries prevention [Bellini et al., 1981; Nyvad, 2003; Hujoel et al., 2006]. It has also been suggested that toothbrushing has been effective in preventing caries mainly because it brings fluoride into the oral cavity at regular intervals, rather than any particular efficiency in disrupting cariogenic plaque biofilms [ten Cate and Zaura, 2012]. Dietary modification, which requires individuals to restrict their exposure to sucrose and other fermentable substrates, is particularly difficult to achieve in present-day society where cariogenic foods are easily available [Duggal and Van Loveren, 2001]. Furthermore, restriction of sucrose intake alone is unlikely to completely prevent dental caries if frequent intake of other starches persisted [Bradshaw and Lynch, 2013]. Thus, while minimizing the aetiological factors contributing to the disease is critical, additional preventive measures commensurate with individual risk status may still be required in many segments of the population.

Caries Epidemiology

For a long time, dental caries was a pandemic that affected children and adults almost universally. The landmark discovery of fluoride as an agent that could prevent dental caries, and the widespread use of fluoride-based caries-preventive programmes, have been responsible for the significant reductions seen in caries prevalence of developed countries in the latter half of the 20th century [Fejerskov, 2004]. However, the latest Global Burden of Disease report revealed that untreated caries in permanent teeth still remains the most common human disease condition worldwide [Kassebaum et al., 2015]. Doubts have also been expressed on whether the earlier decline in caries prevalence has continued into this new century [Gimenez et al., 2016]. Recent caries prevalence studies in the USA and Australia indicate tooth decay may in fact be increasing again [Dye et al., 2007; Chrisopoulos et al., 2016]. A similar trend has been seen in countries such as Norway and Iceland, which had initially shown the biggest improvements, but are now registering increases in caries experience [Haugejorden and Birkeland, 2005; Agustsdottir et al., 2010]. The flattening of prevalence rates that has occurred even in dentally aware populations where individuals commonly brush their teeth with fluoridated dentifrices is concerning and underscores the need to develop additional caries-preventive measures that are synergistic with or complementary to fluoride.

Fluoride Alone May Not Be Sufficient

While fluoride is a highly effective and economical agent for dental caries prevention and will remain the mainstay of any caries-preventive programme, it must be recognized that in many situations fluoride alone may not be sufficient. Even with regular fluoride use, carious lesions can still develop when there are more than 6 dietary sugar exposures per day [Duggal et al., 2001; Ccahuana-Vasquez et al., 2007]. The ready availability of cariogenic snack foods and drinks in the modern consumer culture can overwhelm the benefits of community water fluoridation and daily use of a fluoride dentifrice. The limit to the repair potential of fluoride could partly explain the reversal of the caries decline being observed in contemporary epidemiological reports from developed countries [Agustsdottir et al., 2010; Dye et al., 2017].

The cariostatic actions of fluoride are largely attributed to its physiochemical ability to inhibit enamel demineralization and enhance remineralization [Cury and Tenuta, 2008]. Recent laboratory studies have confirmed the notion that under appropriate conditions fluoride ions can also influence critical MS virulence factors, significantly reducing acidogenicity, aciduricity, and glucan formation [Domon-Tawaraya et al., 2013; Pandit et al., 2013, 2015]. Metabolome analysis of plaque biofilms has demonstrated that fluoride can repress acid production in vivo too [Takahashi and Washio, 2011]. However, questions still remain on the extent to which these antibacterial mechanisms contribute towards caries-preventive effects. A recent study showed that the brief fluoride exposure from toothpastes or mouthwashes could not sustain anti-acid production activity, with the biofilms recovering acidogenicity over time regardless of the fluoride concentration used [Dang et al., 2016]. Concerns have also arisen regarding the emergence of micro-organisms that are more resistant to the effects of fluoride on microbial metabolism [Mitsuhata et al., 2014].

Preventive approaches that combine fluoride with other protective agents have been advocated to enhance the ability of fluoride to modify biofilms and diminish the cariogenic bacterial challenge [Li et al., 2015]. Fluoride-antimicrobial combinations have been recommended based on the 2-part rationale that fluoride can reduce the critical pH at which dissolution starts, while effective antimicrobial agents can decrease the depth of the Stephan curve pH drop following consumption of fermentable carbohydrates [Øgaard, 2000]. Randomized controlled trials (RCTs) have shown that fluoride-chlorhexidine interventions significantly reduced bacterial load and pH drop from glucose metabolism [Giertsen and Scheie, 1995], and

most importantly, lowered caries increment in high-risk groups [Featherstone et al., 2012]. Systematic reviews have also found high-quality evidence that fluoride-triclosan toothpastes give a small improvement in reducing coronal caries compared to conventional fluoride dentifrices [Blinkhorn et al., 2009; Riley and Lamont, 2013]. However, the use of such broad-spectrum antimicrobial agents may not be ideal, as the aim of antimicrobial treatment in caries prevention should preferably be to modify the plaque biofilm ecology by weakening cariogenic virulence factors rather than eliminating microflora.

An alternative to the fluoride-antimicrobial approach is combining fluoride with agents that promote an overall community-wide microbial shift by encouraging the growth of health-associated bacteria, thereby beneficially rebalancing the biofilm ecology and potentially resulting in better long-term dental caries control. Among such biofilm-modifying oral products, there is now sufficient evidence supporting fluoride-arginine combinations as a new standard of care for caries prevention [ten Cate and Cummins, 2013; Zheng et al., 2015]. Likewise, a dentifrice containing enzymes and proteins was shown to significantly shift the ecology of the oral microbiome resulting in a community with a stronger association to health [Adams et al., 2017].

Caries Risk

Over time, the distribution of caries in the community has changed from a disease that was pandemic in society, to now being endemic in specific risk groups. A cross-sectional study concluded that 75% of the caries-risk burden tends to reside in 25–40% of the population [Macek et al., 2004]. Likewise, there is evidence for genetic differences that exist with respect to caries susceptibility [Bretz et al., 2005; Opal et al., 2015], with not everyone in a population group benefiting to the same extent from traditional caries-preventive programmes [ten Cate, 2013]. The pattern of dental caries has also changed over the years, from a rapidly progressing disease of childhood, to a slowly progressing disease that can occur or persist throughout adulthood and old age [Lagerweij and van Loveren, 2015]. Data from contemporary population studies have revealed that carious lesions are also increasingly localized to specific tooth sites [Anderson, 2002]. In children and adolescents, occlusal surfaces are the sites most likely to have experienced caries [Carvalho, 2014; Carvalho et al., 2016]. The increasing life expectancy of dentition also means that older adults with exposed root surfaces are at greater risk of experiencing root caries during their lifetime [Gati and Vieira, 2011]. With fluoride

known to exert its cariostatic actions primarily on smooth enamel surfaces, susceptible occlusal/root surfaces remain relatively predisposed to acid dissolution [ten Cate, 2009]. Caries-preventive strategies must therefore go beyond conventional methods (personal oral hygiene, fluoride dentifrices, and limiting sugar exposures) to better protect at-risk surfaces in at-risk patients.

Fluoride Safety

The US Centers for Disease Control and Prevention ranked community water fluoridation as one of the 10 great public health achievements of the 20th century. Despite continued broad support within the dental profession worldwide, the polarized debate on the safety of fluorides used for caries prevention still continues in sections of the popular and scientific press. Recent reports can only add to the alarmist picture for sections of the general public who are not informed readers of the scientific literature on this topic. For example, a 2012 systematic review and meta-analysis concluded that high fluoride exposure may lower IQ levels in children [Choi et al., 2012], a finding which attracted media attention and triggered the inclusion of fluoride amongst chemicals classified as developmental neurotoxicants [Grandjean and Landrigan, 2014]. There are however a number of concerns with how the 2012 review was conducted – all selected studies were from China, fluoride exposure came from multiple sources not just drinking water, the definition of “high” fluoride levels varied widely, and the probability of confounding as covariates was not controlled. The authors themselves were extremely conservative, only concluding that “our results support the possibility of adverse effects” advocating future research to evaluate dose-response relations based on individual-level measures of exposure over time. However, these caveats are universally overlooked when studies are reported in the media, and this fans extremist views on the safety of fluoride used for caries prevention.

Additionally, dental fluorosis from excessive intake of fluoride during the period of tooth formation is a persisting concern that is raised by anti-fluoride lobbyists. While emphasizing that fluoride is safe when used at the recommended levels for community water fluoridation or dentifrices, behaviours such as swallowing large amounts of dentifrice are not. In modern times there are also now opportunities for “halo” exposure from various other sources of fluoride, which has triggered downward revision of recommended levels for community water fluoridation (from 0.7–1.2 to 0.7 mg/L), to ensure that the risk for dental fluorosis does not increase [EPA, 2011].

Ecological caries-preventive approaches synergistic with fluoride can potentially allow dental products to be designed with lower concentrations of fluoride and these could be useful for caries prevention in infants and young children, as well as for patients reluctant to use oral care products with high fluoride concentrations.

Ecological Approaches to Caries Prevention

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are a heterogeneous group of molecules with unique antimicrobial characteristics that have great potential for controlling bacterial infections and modifying biofilms. AMPs have a broad range of antibacterial, antiviral, and antifungal activity mediated by selectively interacting electrostatically with negatively charged components of cell membrane phospholipids, resulting in membrane permeabilization and disruption, leading to cell death [Koczulla and Bals, 2003]. To escape the actions of bilayer-disruptive AMPs would entail changing membrane composition and organization, a “costly” process in evolutionary terms, meaning that AMPs have very low resistance rates compared to common antibiotics [Zasloff, 2002]. Besides naturally secreted salivary AMPs (lactoferrin, cathelicidins, histatins, defensins), a number of AMPs have been synthesized in the laboratory, and these include specific anticaries peptides that have shown the potential to modify plaque biofilms and inhibit dental caries.

The specifically targeted antimicrobial peptide (STAMP) is a synthetic fusion peptide with 2 independent functional domains, consisting of a *Streptococcus mutans*-selective “targeting domain” designated as C16, and a “killing domain” designated as G2. C16 is derived from a fragment of the *S. mutans* competence stimulating peptide (CSP), while G2 is derived from a broad-spectrum antimicrobial peptide [Eckert et al., 2012]. C16G2 had antimicrobial mechanisms similar to traditional AMPs, and critically, its membrane-disrupting activity specifically targets *S. mutans* from multispecies biofilms without affecting closely related non-cariogenic oral streptococci [Eckert et al., 2006; Kaplan et al., 2011]. More recently, an in vitro study on human saliva-derived polymicrobial biofilms was able to demonstrate that treatment with C16G2 not only eliminated *S. mutans*, but also resulted in a more benign oral microbial community with increased populations of health-associated bacteria and fewer harmful Gram-negative bacteria [Guo et al., 2015]. C16G2 is recognized by the US Food and Drug Adminis-

tration as an investigational new drug for dental caries prevention and has successfully completed phase II clinical trials, where it was delivered to patients in the form of a dental gel loaded in trays.

Another promising anticaries AMP is a synthetic α -helical antimicrobial decapeptide designated KSL-W, which can selectively destabilize the cell membranes of cariogenic bacteria including *S. mutans*, *S. sobrinus*, and *L. acidophilus* [Na et al., 2007; Leung et al., 2009]. This peptide resists enzymatic degradation in human saliva for 1 h, and the potential use of KSL-W as an antibiofilm agent in a chewing gum formulation has been suggested [Faraj et al., 2007; Na et al., 2007]. More recently, a hydroxyapatite-binding AMP was designed, based on the fusion of specific hydroxyapatite-binding heptapeptide (HBP7) with KSL-W, and this bioconjugate was shown to have improved oral retention and antibacterial efficacy [Huang et al., 2016b]. Other AMPs that have shown in vitro antimicrobial activity against cariogenic bacteria and inhibited oral biofilm formation include a synthetic peptide called L-K6 (derived from the naturally occurring peptide temporin-1CEb), a short synthetic amphiphilic peptide known as 1018, and an amphipathic α -helical peptide containing only 12 amino acids named GH12 [Shang et al., 2014; Wang et al., 2015; Tu et al., 2016].

Limitations of AMPs include their potential toxicity, susceptibility to proteases, high cost of peptide production, and the reduced cationic activity of most AMPs in physiological fluids like saliva. With regard to their use for dental caries prevention, questions still remain on whether anticaries AMPs will be able to function against a background of excessive acid production often seen in high caries-risk individuals [Maltz and Beighton, 2012]. Before any clinical recommendations can be made, it will be essential to test such agents in a caries-conducive oral environment, and to evaluate whether the desired outcome of reduced caries increment in at-risk population groups can be achieved.

Probiotics

The term probiotics refers to “live micro-organisms, which, when administered in adequate amounts, confer a health benefit on the host” [Teughels et al., 2008], and is based on the Noble-prize-winning pioneering work of Metchnikoff [1907] for maintaining a healthy gut flora. This concept of implanting a harmless effector strain into the host’s microflora to maintain or restore a natural microbiome by inhibition of pathogenic micro-organisms is attractive and has also been used to support health-associated microbes or restore diversity in the oral plaque bio-

film. The mechanisms by which probiotics re-establish ecological balance in oral biofilms are not fully understood, but probiotic bacteria are believed to have both local and systemic effects [Meurman, 2005]. The local anti-carries effects may include competitive inhibition with cariogenic bacteria for nutrition or adhesive surfaces [Terai et al., 2015], selective co-aggregation of MS without disturbing other oral flora [Twetman et al., 2009; Lang et al., 2010], and bacteriocin-producing probiotics targeting MS [Burton et al., 2013]. The most commonly used and studied probiotics belong to the *Lactobacillus* and *Bifidobacterium* bacterial genera, although not all their strains have the same efficacy in the inhibition of *S. mutans* growth or biofilm formation [Schwendicke et al., 2017].

Evidence supporting the application of probiotics for preventing dental caries is controversial, with recent reports suggesting potential harmful effects for some probiotic bacterial strains [Gruner et al., 2016]. One of the problems identified in using probiotics for caries prevention is that the commonly available *Lactobacillus* and *Bifidobacterium* probiotic bacteria are themselves acidogenic and aciduric, and could contribute to the caries process if such bacteria are allowed to colonize the oral cavity [Maltz and Beighton, 2012]. Recent in vitro biofilm studies have confirmed this apprehension with different strains such as *Lactobacillus salivarius* W24 [Pham et al., 2009], *Lactobacillus rhamnosus* GG [Schwendicke et al., 2014a], *Bifidobacteria animalis lactis* BB12 [Schwendicke et al., 2014b], *Lactobacillus rhamnosus* LB21 [Fernández et al., 2015], and *Lactobacillus acidophilus* LA-5 [Schwendicke et al., 2017] which have all been shown to lower biofilm pH. In fact, some *Lactobacillus* and *Bifidobacterium* strains have greater cariogenic attributes than even MS [Beighton et al., 2010]. Thus, while displacing *S. mutans* from plaque biofilms may in principle be desirable, substituting them for even more cariogenic bacteria will not be useful [Schwendicke et al., 2014a]. Some of the non-acidogenic alternatives to *Lactobacillus* and *Bifidobacterium* that have shown promising early results include *S. salivarius* M18 [Burton et al., 2013], heat-inactivated BB12 [Schwendicke et al., 2014b], and *Weissella cibaria* CMU [Jang et al., 2016].

Another limitation with the traditional use of gut-associated *Lactobacillus* and *Bifidobacterium* probiotic species to promote oral health is that these non-oral bacterial strains may not efficiently colonize the oral niche, which is vital for the long-term success of probiotics [López-López et al., 2017]. Even the use of a bacteriocin-producing strain of *S. salivarius* may not succeed, as *S. salivarius*, while a typical member of the oral soft tissue

flora, has limited ability to colonize tooth surfaces. However, very recently 2 natural oral commensal species, *Streptococcus dentisani* and *Streptococcus* A12 that were isolated from the supragingival plaque of caries-free individuals, have demonstrated promising probiotic effects against dental caries. Both these “active colonizers” have a double probiotic action, as they can not only inhibit the growth of MS, but also moderate plaque pH through their arginolytic actions [Huang et al., 2016a; López-López et al., 2017].

The current decade has seen a number systematic reviews and meta-analyses evaluating the effectiveness of using traditional gut-associated probiotics for caries prevention. A meta-analysis of studies with surrogate caries markers (MS and/or LB counts) concluded that these probiotics significantly decreased MS counts, but there were insufficient data on whether caries increment was reduced as well [Laleman et al., 2014]. A similar inference was also reached by a qualitative systematic review of probiotic caries studies, concluding that clinical recommendations would be premature without more comprehensive RCTs showing an actual reduction in individual caries experience [Twetman and Keller, 2012]. Relatively few RCTs of oral probiotics have used clinical dental caries indicators to prove the efficacy of probiotics in preventing or treating dental caries [Näse et al., 2001; Stecksén-Blicks et al., 2009; Burton et al., 2013; Taipale et al., 2013]. A comprehensive systematic review utilizing evidence from these RCTs concluded that current evidence is insufficient for recommending probiotics in controlling dental caries [Gruner et al., 2016].

Taken together, currently available data indicate that while traditional probiotic bacteria may have a beneficial effect on the gut flora and systemic health, a beneficial and clinically significant effect on the oral flora is yet to be demonstrated with sufficient rigor. However, observations from 2 major clinical trials in children [Näse et al., 2001; Stecksén-Blicks et al., 2009] support the intriguing concept of a “metabolic domino effect,” with reductions in caries risk seen to be accompanied by improvements in general health. Particularly promising for caries prevention is the move away from gut-associated probiotic bacteria to resident oral probiotic strains, such as the 2 *S. mutans*-antagonistic bacterial species *S. dentisani* and A12. Further evidence on the ability of these oral probiotics to inhibit dental caries is awaited with interest.

Prebiotics

The prebiotic approach involves feeding resident microbiota with specific nutrients to create conditions that

favour the growth and dominance of healthy bacteria in the biofilm. The nutritional stimulation of endogenous beneficial oral flora to restore microbial balance and promote oral health has been validated in mixed species models [Slomka et al., 2017]. Oral prebiotic substrates that are especially valuable to prevent caries include arginine, arginine-rich peptides, and urea, as these foods when metabolized create alkalizing effects that counteract the acidogenic environment created by cariogenic bacteria. Many commensal bacteria are able to use arginine or urea to generate ammonia by the arginine deaminase system or urease enzymes, respectively [Bradshaw and Marsh, 1998; Nascimento et al., 2009]. Multiple studies have shown that bacterial production of alkaline metabolites such as ammonia can play a major role in biofilm pH homeostasis and beneficially alter the de-/remineralization equilibrium [Burne and Marquis, 2000; Burne et al., 2012; Nascimento et al., 2014]. A substantial body of evidence from microbiological, genetic, biochemical analyses, and clinical studies has now accumulated confirming that the modulation of the alkaligenic potential of dental biofilms is a promising strategy for caries control [Liu et al., 2012].

The preventive potential of oral alkali production has resulted in the development of commercial oral care products that utilize arginine to promote a healthy resident oral microbiome. In vitro biofilm experiments found that fluoride-arginine combinations synergistically inhibited *S. mutans* but enriched *S. sanguinis* growth within multispecies biofilms, while maintaining a “streptococcal pressure” against the potential growth of oral anaerobe *Porphyromonas gingivalis* in the alkalized biofilm [Zheng et al., 2015]. Fluoride-arginine combinations can also suppress exopolysaccharide production, thus targeting another critical virulence factor for cariogenic biofilms [Zheng et al., 2015]. Human in situ studies and several double-blinded RCTs using a fluoride dentifrice containing 1.5% arginine and an insoluble calcium compound have shown significantly greater protection against carious lesions than a fluoride dentifrice alone [Cantore et al., 2013; Kraivaphan et al., 2013; Srisilapanan et al., 2013; Yin et al., 2013; Petersen et al., 2015]. A systematic review and meta-analysis of the anticaries effects of arginine-containing dentifrice formulations concluded that arginine products provided a superior preventive effect over matched formulations containing fluoride alone [Li et al., 2015]. Other authors have been more conservative in their conclusions, either citing insufficient evidence in support of a caries-preventive effect for arginine, or expressing concerns over the higher cost of arginine-fluo-

ride dentifrices versus any additional caries-preventive effect these may provide [Ástvaldsdóttir et al., 2016]. However, the preponderance of evidence does seem to suggest that the arginolysis is an effective approach to improve oral health and balance the microbial ecology.

Sugar Polyols

Sucrose has been designated as the “arch criminal” in the caries process for a long time [Newbrun, 1967], and the search for alternative non-fermentable sweeteners has attracted much attention. Data collected from in vitro and in vivo studies indicate that such sugar substitutes can exhibit potential anticaries effects through a number of different mechanisms [Matsukubo and Takazoe, 2006]. Xylitol, a naturally occurring 5-carbon sugar polyol, is the non-nutritive sweetener that has been most extensively researched over the past 4 decades for its potential cariostatic effects. Xylitol inhibits MS growth by disrupting their energy production processes, leading to a futile energy cycle and cell death [Martinen et al., 2012]. Although not all MS strains were inhibited by xylitol in this manner, even xylitol-resistant bacteria were found to be less virulent after xylitol treatment [Trahan, 1995]. The predominant delivery vehicle for xylitol has been chewing gums, with xylitol dentifrices, candies, lozenges, and mouthrinses also having been used with varying degrees of success. A substantial body of evidence suggests that 5–6 g of xylitol per day delivered over 3 exposures are needed for worthwhile anticaries effects [Milgrom et al., 2009]. Among other sugar polyols, erythritol has been attracting increasing attention as it has been shown to be more effective than xylitol and sorbitol, and importantly, its anticaries effects were shown to persist for up to 3 years [Honkala et al., 2014; de Cock et al., 2016; Falony et al., 2016].

Despite an immense body of literature, the caries-preventive effects of xylitol products remain inconclusive because of inconsistent study outcomes. While numerous studies have indicated xylitol has beneficial effects on surrogate end points (MS levels, plaque pH, acid production), evidence for worthwhile reductions in caries experience remains equivocal, and there is a need for more double-blind placebo-controlled RCTs, focussing on optimal dosage, delivery vehicle, and possible synergism with other preventive agents [Twetman, 2009; Milgrom et al., 2012]. In fact, the more recent data conclude that there is limited evidence to show xylitol is effective in the fight against dental caries. A double-blind cluster RCT using xylitol gummy bears found that polyol consumption did not provide any additional benefit over other car-

ies-preventive measures [Lee et al., 2015]. Recent Cochrane systematic reviews also found only very-low- to low-quality evidence on xylitol effectiveness which was not sufficient to determine whether xylitol-containing products can prevent caries in infants, children or adults [Duane, 2015; Riley et al., 2015]. Other issues that limit the usefulness of sugar polyols include their high costs and low compliance in high-risk patients because of the need for daily long-term use [Gold, 2016].

Quorum-Sensing Targets

Another approach that may maintain and support a healthy oral plaque ecology is to interfere with the fundamental cell-cell communications system between biofilm bacteria. This process of quorum sensing (QS) is mediated through small diffusible hormone-like molecules (pheromones) and their specific receptors. For MS, the stress-dependent QS system is primarily comprised of the CSP and its ComD/ComE 2-component signal transduction system for communication between biofilm cells of the same species, while interspecies signalling is mediated via the autoinducer-2 molecule produced by LuxS [Senadheera and Cvitkovitch, 2008]. The CSP-mediated QS system in *S. mutans* affects biofilm formation, acidogenicity, aciduricity, genetic transformation, bacteriocin production, stress response, and the ability to produce persister phenotypes [Leung et al., 2015]. Targeting QS signalling pathways could provide a promising avenue in the development of novel therapeutics to alter cariogenic biofilms. As QS is not directly involved in processes essential for bacterial growth, targeting QS will allow less virulent bacteria to remain in the biofilm and will also not impose selective pressures that can lead to the development of antibiotic resistance [Rasmussen and Givskov, 2006].

Interfering with CSP signalling systems has been shown to inactivate a wide range of bacteriocins and mutacins that play an important role in the sustained existence of *S. mutans* in the dental plaque [Cvitkovitch et al., 2003; Qi et al., 2004]. The addition of an exogenous CSP can disrupt signalling events of *S. mutans* and induce cell death [Qi et al., 2005]. Another novel QS-modifying compound, 3-Oxo-N, was seen to significantly minimize lactic acid accumulation without affecting biofilm growth even in the presence of fermentable sugars, representing a promising agent for maintaining a healthy, non-cariogenic microbial ecology in dental plaque [Janus et al., 2016].

Natural Products

Natural products include secondary metabolites or phytochemicals derived from plants, fruits, herbs, or

spices. They offer a rich source of structurally diverse molecules with a wide range of biological activities and could prove useful as alternative or adjunctive anticaries agents [Jeon et al., 2011]. Potential cariostatic mechanisms identified include inhibition of bacterial growth or acid production, inhibition of glucan synthesis by interfering with glucosyltransferase (Gtf) activity, and inhibition of bacterial adhesion [Ferrazzano et al., 2011; Jeon et al., 2011].

Polyphenols from propolis (apigenin and *tt*-farnesol) and cranberry proanthocyanidins have been shown to exert useful ecological effects on the plaque biofilm. Apigenin is a potent inhibitor of water-insoluble glucan synthesis, while *tt*-farnesol disrupts *S. mutans* membrane permeability and acid production [Koo and Jeon, 2009]. An animal study found a combination of these 2 phytochemicals with fluoride suppressed dental caries without affecting the viability of normal oral flora, being as potent as a fluoride-chlorhexidine control in caries inhibition, but without the broad antibacterial action of the control [Koo et al., 2005]. Similarly, cranberry proanthocyanidins, which lack significant biocidal activity, can modify plaque biofilms by reducing acidogenicity and glucan synthesis, and these surrogate end points were also translated into cariostatic effects in vivo [Koo et al., 2010]. A number of other polyphenol compounds have been found to be effective in killing *S. mutans*, with the minimal inhibitory concentrations of some bioactive molecules like xanthorrhizol (from *Curcuma xanthorrhiza*) or macelignan (from *Myristica fragrans*) almost comparable to chlorhexidine [Hwang et al., 2000; Chung et al., 2006]. While most of the tested anticaries phytochemicals showed growth-inhibitory or anti-adhesive effects, a potentially interesting natural agent in caries prevention is *Galla chinensis*, which was able to beneficially regulate the de-/remineralization balance of dental hard tissues [Cheng et al., 2015].

An analysis of how phytochemical research has impacted oral care in the period from 2000 to 2015 found that despite many in vitro, in vivo, and clinical studies testing natural products derived from plants, only 11% of studies were in phase IV clinical trials [Freires and Rosalen, 2016]. Similar conclusions were reached in a systematic review of the anticaries effects of essential oils and their isolated constituents, which found that most studies were conducted in the laboratory, and did not provide botanical characterization or compositional data on the natural product being tested [Freires et al., 2015]. Natural products remain a largely unexplored source of effective and non-toxic antibiofilm molecules that could potential-

ly be used in combination with fluoride as useful alternatives to traditional microbiocides like chlorhexidine or triclosan [Jeon et al., 2011]. However, future research needs to focus on translational approaches to advance the development of effective anticaries products containing phytochemicals or essential oils.

Replacement Therapy with “Designer” Bacteria

Modifying dental plaque by replacing *S. mutans*, a member of the normal microbiota, with a less virulent effector strain has been an established concept for many years [Hillman, 1978]. The rationale for bacterial replacement therapy against dental caries is that relatively avirulent strains of MS are most likely to occupy the same ecological niche in plaque as their more cariogenic counterparts thereby reducing the overall cariogenicity of the plaque biofilm [Sun et al., 2009].

A number of “designer” bacteria have been studied for bacteriotherapy against cariogenic biofilms including a glucan synthesis-defective mutant of *S. mutans*, variants of *S. salivarius* (TOVE-R), and a recombinant alkali-generating ureolytic *S. mutans* strain [Tanzer et al., 1974, 1985; Clancy et al., 2000]. The most extensive research in using genetically modified bacteria for preventing dental caries used a wild-type *S. mutans* strain that naturally produces an antibiotic called mutacin 1140 capable of killing all other strains of *S. mutans* [Hillman, 2002]. This strain was genetically modified by deleting the open reading frame for lactate dehydrogenase, to yield a viable strain called BCS3-L1 that still produced wild-type levels of mutacin 1140, but notably produced no lactic acid [Hillman et al., 2000]. In laboratory and animal models, the BCS3-L1 strain proved to have significantly reduced cariogenic potential. It persistently and pre-emptively colonized tooth sites normally occupied by wild-type *S. mutans* strains, with no reported adverse effects [Hillman et al., 2000]. To overcome safety issues and to enable the altered strain to be implanted into human oral biofilms for clinical trials, additional genetic modifications of BCS3-L1 were made, which could facilitate its rapid elimination should an adverse event manifest itself. This strain was designated as A2JM and was extensively tested to assure its safe use in phase I clinical trials [Hillman et al., 2007].

It has also been suggested that hypocariogenic strains exhibiting only defects in acid production are unlikely to compete successfully with wild-type strains for initial plaque locations, and on this principle, an *S. mutans* strain that was deficient in the *gcrR* gene was genetically engineered for bacterial replacement therapy [Sun et al.,

2009; Pan et al., 2013]. The *gcrR* gene functions as a negative transcriptional regulator of the *gbpC* gene, which encodes the glucan-binding lectin, an adhesin that is ubiquitous on *S. mutans* surfaces and plays an important role in initial bacterial aggregation and adhesion to tooth surfaces. The MS-*gcrR*-def mutant bacteria showed reduced acid production, out-competed wild *S. mutans* strains with its strong early colonization ability, and lowered caries incidence in vivo [Pan et al., 2013].

Modulation of oral plaque biofilms with genetically engineered “designer” bacteria has great potential through fostering a healthy oral environment, which prevents the dominance of cariogenic bacteria. A single treatment regimen could lead to persistent colonization by the effector strain affording lifelong protection, with minimal need for patient compliance. Whilst there have been encouraging results with genetically modified strains, the concept of replacement therapy needs to be tested for effectiveness in highly cariogenic environments. Even if successful, the widespread acceptance of genetically engineered “designer” bacteria may prove to be difficult for emotional, ethical, and legal reasons.

Other Approaches

In addition to the strategies discussed above, several alternative approaches for modifying plaque biofilm ecology are under investigation including using compounds that specifically affect bacterial virulence proteins [Horst et al., 2012], calcium phosphate-osteopontin particles that can inhibit biofilm formation and reduce the fall in pH without affecting bacterial viability [Schlafer et al., 2017], nanoparticles [Allaker and Douglas, 2015], graphene oxide [He et al., 2015], and ceramic water [Nomura et al., 2017].

Conclusions

There is no doubt that fluoride will continue to be the mainstay of any caries prevention protocol as it still remains the most effective and economical protective agent against dental caries. However, fluoride alone may not offer complete protection against the disease, and it is generally recognized that the effectiveness of fluoride could be enhanced when combined with additional cariostatic agents [NIH, 2001]. Moreover, current paradigms emphasize the importance of maintaining a healthy and stable oral plaque biofilm for long-term disease control. One way to do this is to limit or exclude refined sugars from the diet; however, within the constraints of present-

day consumer culture, behavioural dietary changes are difficult to achieve and sustain. Adopting ecological preventive measures can help in correcting the disturbed plaque ecology and drive the advent and persistence of a symbiotic oral microbiome. These could be valuable tools in achieving long-term dental caries control, allowing the clinician to shift to a biological model for the management of the disease.

It is imperative that the effectiveness of ecological preventive approaches be evaluated for success in individuals who consume a conventional diet containing a fairly high level of sugars before any clinical recommendations are made [Beighton, 2009]. Furthermore, rather than surrogate end points like lower MS levels or reduced acid production, the critically important outcome for all new

caries-preventive measures will be whether they can ensure a significant reduction in individual caries experience.

Disclosure Statement

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Author Contributions

N.P. conceived and drafted the manuscript with support and input from B.S. L.J.W. critically reviewed and revised the manuscript. N.P., B.S., and L.J.W. approved the final manuscript as submitted.

References

- Adams SE, Arnold D, Murphy B, Carroll P, Green AK, Smith AM, Marsh PD, Chen T, Marriott RE, Brading MG: A randomised clinical study to determine the effect of a toothpaste containing enzymes and proteins on plaque oral microbiome ecology. *Sci Rep* 2017;7:43344.
- Agustsdottir H, Gudmundsdottir H, Eggertsson H, Jonsson SH, Gudlaugsson JO, Saemundsson SR, Eliasson ST, Arnadottir IB, Holbrook WP: Caries prevalence of permanent teeth: a national survey of children in Iceland using ICDAS. *Community Dent Oral Epidemiol* 2010;38:299–309.
- Allaker RP, Douglas ICW: Non-conventional therapeutics for oral infections. *Virulence* 2015;6:196–207.
- Anderson M: Risk assessment and epidemiology of dental caries: review of the literature. *Pediatr Dent* 2002;24:377–385.
- Ástvaldsdóttir Á, Naimi-Akbar A, Davidson T, Brolund A, Lintamo L, Attergren Granath A, Tranæus S, Östlund P: Arginine and caries prevention: a systematic review. *Caries Res* 2016;50:383–393.
- Beighton D: Can the ecology of the dental biofilm be beneficially altered? *Adv Dent Res* 2009;21:69–73.
- Beighton D, Al-Haboubi M, Mantzourani M, Gilbert SC, Clark D, Zoitopoulos L, Gallagher JE: Oral *Bifidobacteria*: caries-associated bacteria in older adults. *J Dent Res* 2010;89:970–974.
- Bellini HT, Arneberg P, von der Fehr FR: Oral hygiene and caries. A review. *Acta Odontol Scand* 1981;39:257–265.
- Blinkhorn A, Bartold PM, Cullinan MP, Madden TE, Marshall RI, Raphael SL, Seymour GJ: Is there a role for triclosan/copolymer toothpaste in the management of periodontal disease? *Br Dent J* 2009;207:117–125.
- Bradshaw DJ, Lynch RJM: Diet and the microbial aetiology of dental caries: New paradigms. *Int Dent J* 2013;63:64–72.
- Bradshaw DJ, Marsh PD: Analysis of pH-driven disruption of oral microbial communities in vitro. *Caries Res* 1998;32:456–462.
- Bretz WA, Corby PM, Schork NJ, Robinson MT, Coelho M, Costa S, Melo Filho MR, Weyant RJ, Hart TC: Longitudinal analysis of heritability for dental caries traits. *J Dent Res* 2005;84:1047–1051.
- Burne RA, Marquis RE: Alkali production by oral bacteria and protection against dental caries. *FEMS Microbiol Lett* 2000;193:1–6.
- Burne RA, Zeng L, Ahn SJ, Palmer SR, Liu Y, Lefebure T, Stanhope MJ, Nascimento MM: Progress dissecting the oral microbiome in caries and health. *Adv Dent Res* 2012;24:77–80.
- Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, Hale JDF, Wescombe PA: Influence of the probiotic *Streptococcus salivarius* strain M18 on indices of dental health in children: a randomized double-blind, placebo-controlled trial. *J Med Microbiol* 2013;62:875–884.
- Cantore R, Petrou I, Lavender S, Santarpia P, Liu Z, Gittins E, Vandeven M, Cummins D, Sullivan R, Utgikar N: In situ clinical effects of new dentifrices containing 1.5% arginine and fluoride on enamel de- and remineralization and plaque metabolism. *J Clin Dent* 2013;24(Spec No A):A32–A44.
- Carvalho JC: Caries process on occlusal surfaces: evolving evidence and understanding. *Caries Res* 2014;48:339–346.
- Carvalho JC, Dige I, Machiulskiene V, Qvist V, Bakhshandeh A, Fatturi-Parolo C, Maltz M: Occlusal caries: Biological approach for its diagnosis and management. *Caries Res* 2016;50:527–542.
- Casamassimo PS, Thikkurissy S, Edelstein BL, Maiorini E: Beyond the dmft: the human and economic cost of early childhood caries. *J Am Dent Assoc* 2009;140:650–657.
- Caufield PW, Dasanayake AP, Li Y: The antimicrobial approach to caries management. *J Dent Educ* 2001;65:1091–1095.
- Ccahuana-Vasquez RA, Tabchoury CP, Tenuta LM, Del Bel Cury AA, Vale GC, Cury JA: Effect of frequency of sucrose exposure on dental biofilm composition and enamel demineralization in the presence of fluoride. *Caries Res* 2007;41:9–15.
- Cheng L, Li J, He L, Zhou X: Natural products and caries prevention. *Caries Res* 2015;49(suppl 1):38–45.
- Choi AL, Sun G, Zhang Y, Grandjean P: Developmental fluoride neurotoxicity: a systematic review and meta-analysis. *Environ Health Perspect* 2012;120:1362–1368.
- Chrisopoulos S, Harford JE, Ellershaw A: Oral health and dental care in Australia: key facts and figures 2015. Cat. No. Den 229. Canberra, Australian Institute of Health and Welfare, 2016.
- Chung JY, Choo JH, Lee MH, Hwang JK: Anticariogenic activity of macelignan isolated from *Myristica fragrans* (nutmeg) against *Streptococcus mutans*. *Phytomedicine* 2006;13:261–266.
- Clancy KA, Pearson S, Bowen WH, Burne RA: Characterization of recombinant, ureolytic *Streptococcus mutans* demonstrates an inverse relationship between dental plaque ureolytic capacity and cariogenicity. *Infect Immun* 2000;68:2621–2629.
- Cury JA, Tenuta LMA: How to maintain a cariostatic fluoride concentration in the oral environment. *Adv Dent Res* 2008;20:13–16.

- Cvitkovitch DG, Li YH, Ellen RP: Quorum sensing and biofilm formation in streptococcal infections. *J Clin Invest* 2003;112:1626–1632.
- Dang MH, Jung JE, Lee DW, Song KY, Jeon JG: Recovery of acid production in *Streptococcus mutans* biofilms after short-term fluoride treatment. *Caries Res* 2016;50:363–371.
- De Cock P, Mäkinen K, Honkala E, Saag M, Ken-nepohl E, Eapen A: Erythritol is more effective than xylitol and sorbitol in managing oral health endpoints. *Int J Dent* 2016;2016:9868421.
- Devine DA, Marsh PD, Meade J: Modulation of host responses by oral commensal bacteria. *J Oral Microbiol* 2015;7:26941.
- Domon-Tawaraya H, Nakajo K, Washio J, Ashi-zawa T, Ichino T, Sugawara H, Fukumoto S, Takahashi N: Divalent cations enhance fluo-ride binding to *Streptococcus mutans* and *Streptococcus sanguinis* cells and subsequently inhibit bacterial acid production. *Caries Res* 2013;47:141–149.
- Duane B: Xylitol and caries prevention. *Evid Based Dent* 2015;16:37–38.
- Duggal MS, Toumba KJ, Amaechi BT, Kowash MB, Higham SM: Enamel demineralization in situ with various frequencies of carbohydrate consumption with and without fluoride toothpaste. *J Dent Res* 2001;80:1721–1724.
- Duggal MS, van Loveren C: Dental considerations for dietary counselling. *Int Dent J* 2001;51:408–412.
- Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, Eke PI, Beltran-Aguilar ED, Horowitz AM, Li CH: Trends in oral health status: United States, 1988–1994 and 1999–2004. *Vital Health Stat* 11 2007;1–92.
- Dye BA, Vargas CM, Fryar CD, Ramos-Gomez F, Isman R: Oral health status of children in Los Angeles County and in the United States, 1999–2004. *Community Dent Oral Epidemiol* 2017;45:135–144.
- Eckert R, He J, Yarbrough DK, Qi F, Anderson MH, Shi W: Targeted killing of *Streptococcus mutans* by a pheromone-guided “smart” anti-microbial peptide. *Antimicrob Agents Chemother* 2006;50:3651–3657.
- Eckert R, Sullivan R, Shi W: Targeted antimicrobial treatment to re-establish a healthy microbial flora for long-term protection. *Adv Dent Res* 2012;24:94–97.
- EPA: EPA and HHS announce new scientific assessments and actions on fluoride: agencies working together to maintain benefits of preventing tooth decay while preventing excessive exposure. 2011. <http://yosemite.epa.gov/opa/admpress.nsf/bd4379a92ceceac8525735900400c27/86964af577c37ab285257811005a8417!OpenDocument>.
- Falony G, Honkala S, Runnel R, Olak J, Nömmela R, Russak S, Saag M, Mäkinen PL, Mäkinen K, Vahlberg T, Honkala E: Long-term effect of erythritol on dental caries development during childhood: a posttreatment survival analysis. *Caries Res* 2016;50:579–588.
- Faraj JA, Dorati R, Schoubben A, Worthen D, Sel-min F, Capan Y, Leung K, DeLuca PP: Development of a peptide-containing chewing gum as a sustained release antiplaque antimicrobial delivery system. *AAPS Pharm Sci Tech* 2007;8:26.
- Featherstone JD, White JM, Hoover CI, Rapozo-Hilo M, Weintraub JA, Wilson RS, Zhan L, Gansky SA: A randomized clinical trial of anticaries therapies targeted according to risk assessment (caries management by risk assessment). *Caries Res* 2012;46:118–129.
- Fejerskov O: Changing paradigms in concepts on dental caries: consequences for oral health care. *Caries Res* 2004;38:182–191.
- Fernández CE, Giacaman RA, Tenuta LM, Cury JA: Effect of the probiotic *Lactobacillus rham-nosus* LB21 on the cariogenicity of *Streptococ-cus mutans* UA159 in a dual-species biofilm model. *Caries Res* 2015;49:583–590.
- Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A: Plant polyphenols and their anti-cariogenic properties: a review. *Molecules* 2011;16:1486–1507.
- Freires I, Denny C, Benso B, de Alencar S, Rosalen P: Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review. *Molecules* 2015;20:7329.
- Freires IA, Rosalen PL: How natural product research has contributed to oral care product development? A critical view. *Pharm Res* 2016;33:1311–1317.
- Gati D, Vieira AR: Elderly at greater risk for root caries: a look at the multifactorial risks with emphasis on genetics susceptibility. *Int J Dent* 2011;2011:647168.
- Giertsens E, Scheie AA: Effects of chlorhexidine-fluoride mouthrinses on viability, acidogenic potential, and glycolytic profile of established dental plaque. *Caries Res* 1995;29:181–187.
- Gimenez T, Bispo BA, Souza DP, Vigano ME, Wanderley MT, Mendes FM, Bonecker M, Braga MM: Does the decline in caries prevalence of Latin American and Caribbean children continue in the new century? Evidence from systematic review with meta-analysis. *PLoS One* 2016;11:e0164903.
- Gold J: Consumption of xylitol gummy bears may not provide additional caries prevention for school children. *J Evid Based Dent Pract* 2016;16:70–72.
- Grandjean P, Landrigan PJ: Neurobehavioural effects of developmental toxicity. *Lancet Neurol* 2014;13:330–338.
- Gruner D, Paris S, Schwendicke F: Probiotics for managing caries and periodontitis: systematic review and meta-analysis. *J Dent* 2016;48:16–25.
- Guo L, McLean JS, Yang Y, Eckert R, Kaplan CW, Kyme P, Sheikh O, Varnum B, Lux R, Shi W, He X: Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc Natl Acad Sci USA* 2015;112:7569–7574.
- Haugejorden O, Birkeland JM: Analysis of the ups and downs of caries experience among Norwegian children aged five years between 1997 and 2003. *Acta Odontol Scand* 2005;63:115–122.
- He J, Zhu X, Qi Z, Wang C, Mao X, Zhu C, He Z, Li M, Tang Z: Killing dental pathogens using antibacterial graphene oxide. *ACS Appl Mater Interfaces* 2015;7:5605–5611.
- He X, Hu W, He J, Guo L, Lux R, Shi W: Community-based interference against integration of *Pseudomonas aeruginosa* into human salivary microbial biofilm. *Mol Oral Microbiol* 2011;26:337–352.
- Hillman JD: Lactate dehydrogenase mutants of *Streptococcus mutans*: isolation and preliminary characterization. *Infect Immun* 1978;21:206–212.
- Hillman JD: Genetically modified *Streptococcus mutans* for the prevention of dental caries. *Antonie Van Leeuwenhoek* 2002;82:361–366.
- Hillman JD, Brooks TA, Michalek SM, Harmon CC, Snoep JL, van der Weijden CC: Construction and characterization of an effector strain of *Streptococcus mutans* for replacement therapy of dental caries. *Infect Immun* 2000;68:543–549.
- Hillman JD, Mo J, McDonell E, Cvitkovitch D, Hillman CH: Modification of an effector strain for replacement therapy of dental caries to enable clinical safety trials. *J Appl Microbiol* 2007;102:1209–1219.
- Honkala S, Runnel R, Saag M, Olak J, Nömmela R, Russak S, Mäkinen PL, Vahlberg T, Falony G, Mäkinen K, Honkala E: Effect of erythritol and xylitol on dental caries prevention in children. *Caries Res* 2014;48:482–490.
- Honorio HM, Rios D, Santos CF, Buzalaf MA, Machado MA: Influence of dental plaque on human enamel erosion: in situ/ex vivo study. *Oral Health Prev Dent* 2010;8:179–184.
- Horst JA, Pieper U, Sali A, Zhan L, Chopra G, Samudrala R, Featherstone JDB: Strategic protein target analysis for developing drugs to stop dental caries. *Adv Dent Res* 2012;24:86–93.
- Huang X, Palmer S, Ahn S-J, Richards VP, Williams ML, Nascimento MM, Burne RA: Characterization of a highly arginolytic *Streptococcus* species that potentially antagonizes *Streptococcus mutans*. *Appl Environ Microbiol* 2016a;82:2187–2201.
- Huang ZB, Shi X, Mao J, Gong SQ: Design of a hydroxyapatite-binding antimicrobial peptide with improved retention and antibacterial efficacy for oral pathogen control. *Sci Rep* 2016b;6:38410.
- Hujoel PP, Cunha-Cruz J, Banting DW, Loesche WJ: Dental flossing and interproximal caries: a systematic review. *J Dent Res* 2006;85:298–305.
- Hwang JK, Shim JS, Pyun YR: Antibacterial activity of xanthorrhizol from *Curcuma xanthorrhiza* against oral pathogens. *Fitoterapia* 2000;71:321–323.

- Jang H-J, Kang M-S, Yi S-H, Hong J-Y, Hong S-P: Comparative study on the characteristics of *Weissella cibaria* CMU and probiotic strains for oral care. *Molecules* 2016;21:1752.
- Janus MM, Crielaard W, Zaura E, Keijser BJ, Brandt BW, Krom BP: A novel compound to maintain a healthy oral plaque ecology in vitro. *J Oral Microbiol* 2016;8:10.
- Jeon JG, Rosalen PL, Falsetta ML, Koo H: Natural products in caries research: current (limited) knowledge, challenges and future perspective. *Caries Res* 2011;45:243–263.
- Kaplan CW, Sim JH, Shah KR, Kolesnikova-Kaplan A, Shi W, Eckert R: Selective membrane disruption: mode of action of C16G2, a specifically targeted antimicrobial peptide. *Antimicrob Agents Chemother* 2011;55:3446–3452.
- Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W: Global burden of untreated caries: a systematic review and metaregression. *J Dent Res* 2015;94:650–658.
- Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade WG, Zaura E: The oral microbiome – an update for oral healthcare professionals. *Br Dent J* 2016;221:657–666.
- Kleinberg I: A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to *Streptococcus mutans* and the specific-plaque hypothesis. *Crit Rev Oral Biol Med* 2002;13:108–125.
- Kocuzulla AR, Bals R: Antimicrobial peptides: current status and therapeutic potential. *Drugs* 2003;63:389–406.
- Koo H, Bowen WH: *Candida albicans* and *Streptococcus mutans*: a potential synergistic alliance to cause virulent tooth decay in children. *Future Microbiol* 2014;9:1295–1297.
- Koo H, Duarte S, Murata RM, Scott-Anne K, Greigore S, Watson GE, Singh AP, Vorsa N: Influence of cranberry proanthocyanidins on formation of biofilms by *Streptococcus mutans* on saliva-coated apatitic surface and on dental caries development in vivo. *Caries Res* 2010;44:116–126.
- Koo H, Jeon JG: Naturally occurring molecules as alternative therapeutic agents against cariogenic biofilms. *Adv Dent Res* 2009;21:63–68.
- Koo H, Schobel B, Scott-Anne K, Watson G, Bowen WH, Cury JA, Rosalen PL, Park YK: Apigenin and tt-farnesol with fluoride effects on *S. mutans* biofilms and dental caries. *J Dent Res* 2005;84:1016–1020.
- Kraivaphan P, Amornchat C, Triratana T, Mateo LR, Ellwood R, Cummins D, DeVizio W, Zhang YP: Two-year caries clinical study of the efficacy of novel dentifrices containing 1.5% arginine, an insoluble calcium compound and 1,450 ppm fluoride. *Caries Res* 2013;47:582–590.
- Lagerweij MD, van Loveren C: Declining caries trends: are we satisfied? *Curr Oral Health Rep* 2015;2:212–217.
- Laleman I, Dettailleur V, Slot DE, Slomka V, Quirynen M, Teughels W: Probiotics reduce mutans streptococci counts in humans: a systematic review and meta-analysis. *Clin Oral Investig* 2014;18:1539–1552.
- Lang C, Bottner M, Holz C, Veen M, Ryser M, Reindl A, Pompejus M, Tanzer JM: Specific *Lactobacillus/mutans streptococcus* co-aggregation. *J Dent Res* 2010;89:175–179.
- Lee W, Spiekerman C, Heima M, Eggertsson H, Ferretti G, Milgrom P, Nelson S: The effectiveness of xylitol in a school-based cluster-randomized clinical trial. *Caries Res* 2015;49:41–49.
- Leung V, Ajdic D, Koyanagi S, Levesque CM: The formation of *Streptococcus mutans* persists induced by the quorum-sensing peptide pheromone is affected by the *LexA* regulator. *J Bacteriol* 2015;197:1083–1094.
- Leung KP, Abercrombie JJ, Campbell TM, Gilmore KD, Bell CA, Faraj JA, DeLuca PP: Antimicrobial peptides for plaque control. *Adv Dent Res* 2009;21:57–62.
- Li J, Huang Z, Mei L, Li G, Li H: Anti-caries effect of arginine-containing formulations in vivo: a systematic review and meta-analysis. *Caries Res* 2015;49:606–617.
- Liu Y-L, Nascimento M, Burne RA: Progress toward understanding the contribution of alkali generation in dental biofilms to inhibition of dental caries. *Int J Oral Sci* 2012;4:135–140.
- López-López A, Camelo-Castillo A, Ferrer MD, Simon-Soro Á, Mira A: Health-associated niche inhabitants as oral probiotics: the case of *Streptococcus dentisani*. *Front Microbiol* 2017;8:379.
- Macek MD, Heller KE, Selwitz RH, Manz MC: Is 75 percent of dental caries really found in 25 percent of the population? *J Public Health Dent* 2004;64:20–25.
- Maltz M, Beighton D: Multidisciplinary research agenda for novel antimicrobial agents for caries prevention and treatment. *Adv Dent Res* 2012;24:133–136.
- Martinen AM, Ruas-Madiedo P, Hidalgo-Cantabrana C, Saari MA, Ihalin RA, Soderling EM: Effects of xylitol on xylitol-sensitive versus xylitol-resistant *Streptococcus mutans* strains in a three-species in vitro biofilm. *Curr Microbiol* 2012;65:237–243.
- Marsh PD, Head DA, Devine DA: Ecological approaches to oral biofilms: control without killing. *Caries Res* 2015;49(suppl 1):46–54.
- Marsh PD, Martin MV: The resident oral microflora; in Marsh PD, Martin VM (eds): *Oral Microbiology*. Woburn, Reed Educational and Professional Publishing, 1999, pp 7–33.
- Matsukubo T, Takazoe I: Sucrose substitutes and their role in caries prevention. *Int Dent J* 2006;56:119–130.
- Metchnikoff E: Lactic acid as inhibiting intestinal putrefaction; in Metchnikoff E, Mitchell PC (eds): *The Prolongation of Life: Optimistic Studies*. London, Heinemann, 1907, pp 161–183.
- Meurman JH: Probiotics: do they have a role in oral medicine and dentistry? *Eur J Oral Sci* 2005;113:188–196.
- Milgrom P, Ly KA, Rothen M: Research findings on xylitol and the development of xylitol vehicles to address public health needs. *Adv Dent Res* 2009;21:10–25.
- Milgrom P, Söderling EM, Nelson S, Chi DL, Nakai Y: Clinical evidence for polyol efficacy. *Adv Dental Res* 2012;24:112–116.
- Mitsuhata C, Puteri MM, Ohara Y, Tatsukawa N, Kozai K: Possible involvement of enolase in fluoride resistance in *Streptococcus mutans*. *Pediatr Dent J* 2014;24:12–16.
- Na DH, Faraj J, Capan Y, Leung KP, DeLuca PP: Stability of antimicrobial decapeptide (KSL) and its analogues for delivery in the oral cavity. *Pharm Res* 2007;24:1544–1550.
- Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, Korpela R, Meurman JH: Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res* 2001;35:412–420.
- Nascimento MM, Browngardt C, Xiaohui X, Klepac-Ceraj V, Paster BJ, Burne RA: The effect of arginine on oral biofilm communities. *Mol Oral Microbiol* 2014;29:45–54.
- Nascimento MM, Gordan VV, Garvan CW, Browngardt CM, Burne RA: Correlations of oral bacterial arginine and urea catabolism with caries experience. *Oral Microbiol Immunol* 2009;24:89–95.
- Newbrun E: Sucrose, the arch criminal of dental caries. *Odontol Rev* 1967;18:373–386.
- NIH: Diagnosis and management of dental caries throughout life. *NIH Consensus Statement* 2001;18:1–23.
- Nomura R, Yoneyama R, Naka S, Otsugu M, Oga-ya Y, Hatakeyama R, Morita Y, Maruo J, Matsumoto-Nakano M, Yamada O, Nakano K: The in vivo inhibition of oral biofilm accumulation and *Streptococcus mutans* by ceramic water. *Caries Res* 2017;51:58–67.
- Nyvad B: The role of oral hygiene; in Fejerskov O, Kidd EAM (eds): *Dental Caries: The Disease and Its Clinical Management*. Copenhagen, Blackwell Munksgaard, 2003, pp 171–177.
- Øgaard B: Oral microbiological changes, long-term enamel alterations due to decalcification, and caries prophylactic aspects; in Brantley WA, Eliades T (eds): *Orthodontic Materials. Scientific and Clinical Aspects*. Stuttgart, Thieme, 2000, pp 123–124.
- Opal S, Garg S, Jain J, Walia I: Genetic factors affecting dental caries risk. *Aust Dent J* 2015;60:2–11.
- Pan W, Mao T, Xu Q-A, Shao J, Liu C, Fan M: A new *gcrR*-deficient *Streptococcus mutans* mutant for replacement therapy of dental caries. *Sci World J* 2013;2013:460202.
- Pandit S, Cai JN, Jung JE, Jeon JG: Effect of 1-minute fluoride treatment on potential virulence and viability of a cariogenic biofilm. *Caries Res* 2015;49:449–457.

- Pandit S, Kim HJ, Song KY, Jeon JG: Relationship between fluoride concentration and activity against virulence factors and viability of a cariogenic biofilm: in vitro study. *Caries Res* 2013;47:539–547.
- Petersen PE, Hunsrisakhun J, Thearmentree A, Pithpornchaiyakul S, Hintao J, Jurgensen N, Ellwood RP: School-based intervention for improving the oral health of children in southern Thailand. *Community Dent Health* 2015;32:44–50.
- Pham LC, van Spanning RJ, Roling WF, Prosperi AC, Terefework Z, Ten Cate JM, Crielaard W, Zaura E: Effects of probiotic *Lactobacillus salivarius* W24 on the compositional stability of oral microbial communities. *Arch Oral Biol* 2009;54:132–137.
- Qi F, Kreth J, Lévesque CM, Kay O, Mair RW, Shi W, Cvitkovitch DG, Goodman SD: Peptide pheromone induced cell death of *Streptococcus mutans*. *FEMS Microbiol Lett* 2005;251:321–326.
- Qi F, Merritt J, Lux R, Shi W: Inactivation of the *ciaH* gene in *Streptococcus mutans* diminishes mutacin production and competence development, alters sucrose-dependent biofilm formation, and reduces stress tolerance. *Infect Immun* 2004;72:4895–4899.
- Rasmussen TB, Givskov M: Quorum sensing inhibitors: a bargain of effects. *Microbiology* 2006;152:895–904.
- Riley P, Lamont T: Triclosan/copolymer containing toothpastes for oral health. *Cochrane Database Syst Rev* 2013;12:CD010514.
- Riley P, Moore D, Ahmed F, Sharif MO, Worthington HV: Xylitol-containing products for preventing dental caries in children and adults. *Cochrane Database Syst Rev* 2015;26:CD010743.
- Schlafer S, Ibsen CJS, Birkedal H, Nyvad B: Calcium-phosphate-osteopontin particles reduce biofilm formation and pH drops in in situ grown dental biofilms. *Caries Res* 2017;51:26–33.
- Schwendicke F, Dörfer C, Kneist S, Meyer-Lueckel H, Paris S: Cariogenic effects of probiotic *Lactobacillus rhamnosus* GG in a dental biofilm model. *Caries Res* 2014a;48:186–192.
- Schwendicke F, Horb K, Kneist S, Dörfer C, Paris S: Effects of heat-inactivated *Bifidobacterium* BB12 on cariogenicity of *Streptococcus mutans* in vitro. *Arch Oral Biol* 2014b;59:1384–1390.
- Schwendicke F, Korte F, Dörfer CE, Kneist S, Fawzy El-Sayed K, Paris S: Inhibition of *Streptococcus mutans* growth and biofilm formation by probiotics in vitro. *Caries Res* 2017;51:87–95.
- Senadheera D, Cvitkovitch DG: Quorum sensing and biofilm formation by *Streptococcus mutans*; in Utsumi R (ed): *Bacterial Signal Transduction: Networks and Drug Targets*. New York, Springer, 2008, pp 178–188.
- Shang D, Liang H, Wei S, Yan X, Yang Q, Sun Y: Effects of antimicrobial peptide L-K6, a temporin-1CEb analog on oral pathogen growth, *Streptococcus mutans* biofilm formation, and anti-inflammatory activity. *Appl Microbiol Biotechnol* 2014;98:8685–8695.
- Slomka V, Hernandez-Sanabria E, Herrero ER, Zaidel L, Bernaerts K, Boon N, Quirynen M, Teughels W: Nutritional stimulation of commensal oral bacteria suppresses pathogens: the prebiotic concept. *J Clin Periodontol* 2017;44:344–352.
- Srisilapanan P, Korwanich N, Yin W, Chuensuwonkul C, Mateo LR, Zhang YP, Cummins D, Ellwood RP: Comparison of the efficacy of a dentifrice containing 1.5% arginine and 1,450 ppm fluoride to a dentifrice containing 1,450 ppm fluoride alone in the management of early coronal caries as assessed using quantitative light-induced fluorescence. *J Dent* 2013;41(suppl 2):29–34.
- Stecksén-Blicks C, Sjöström I, Twetman S: Effect of long-term consumption of milk supplemented with probiotic *Lactobacilli* and fluoride on dental caries and general health in preschool children: A cluster-randomized study. *Caries Res* 2009;43:374–381.
- Sun J-H, Xu Q-A, Fan M-W: A new strategy for the replacement therapy of dental caries. *Med Hypotheses* 2009;73:1063–1064.
- Taipale T, Pienihäkkinen K, Alanen P, Jokela J, Söderling E: Administration of *Bifidobacterium animalis* subsp. *lactis* BB-12 in early childhood: a post-trial effect on caries occurrence at four years of age. *Caries Res* 2013;47:364–372.
- Takahashi N, Nyvad B: Caries ecology revisited: microbial dynamics and the caries process. *Caries Res* 2008;42:409–418.
- Takahashi N, Nyvad B: The role of bacteria in the caries process: ecological perspectives. *J Dent Res* 2011;90:294–303.
- Takahashi N, Washio J: Metabolomic effects of xylitol and fluoride on plaque biofilm in vivo. *J Dent Res* 2011;90:1463–1468.
- Tanner AC, Kressler CA, Faller LL: Understanding caries from the oral microbiome perspective. *J Calif Dent Assoc* 2016;44:437–446.
- Tanzer JM, Freedman ML, Fitzgerald RJ, Larson RH: Diminished virulence of glucan synthesis-defective mutants of *Streptococcus mutans*. *Infect Immun* 1974;10:197–203.
- Tanzer JM, Kurasz AB, Clive J: Inhibition of ecological emergence of mutans streptococci naturally transmitted between rats and consequent caries inhibition by *Streptococcus salivarius* TOVE-R infection. *Infect Immun* 1985;49:76–83.
- Ten Cate JM: The need for antibacterial approaches to improve caries control. *Adv Dent Res* 2009;21:8–12.
- Ten Cate JM: Contemporary perspective on the use of fluoride products in caries prevention. *Br Dent J* 2013;214:161–167.
- Ten Cate JM, Cummins D: Fluoride toothpaste containing 1.5% arginine and insoluble calcium as a new standard of care in caries prevention. *J Clin Dent* 2013;24:79–87.
- Ten Cate JM, Zaura E: The numerous microbial species in oral biofilms: how could antibacterial therapy be effective? *Adv Dent Res* 2012;24:108–111.
- Terai T, Okumura T, Imai S, Nakao M, Yamaji K, Ito M, Nagata T, Kaneko K, Miyazaki K, Okada A, Nomura Y, Hanada N: Screening of probiotic candidates in human oral bacteria for the prevention of dental disease. *PLoS One* 2015;10:e0128657.
- Teughels W, Van Essche M, Sliepen I, Quirynen M: Probiotics and oral healthcare. *Periodontol* 2000 2008;48:111–147.
- Trahan L: Xylitol: a review of its action on mutans streptococci and dental plaque – its clinical significance. *Int Dent J* 1995;45:77–92.
- Tu H, Fan Y, Lv X, Han S, Zhou X, Zhang L: Activity of synthetic antimicrobial peptide GH12 against oral streptococci. *Caries Res* 2016;50:48–61.
- Twetman L, Larsen U, Fiehn NE, Stecksén-Blicks C, Twetman S: Coaggregation between probiotic bacteria and caries-associated strains: an in vitro study. *Acta Odontol Scand* 2009;67:284–288.
- Twetman S: Current controversies – is there merit? *Adv Dent Res* 2009;21:48–52.
- Twetman S, Keller MK: Probiotics for caries prevention and control. *Adv Dent Res* 2012;24:98–102.
- Walsh LJ: New paradigms for assessing caries risk and lesion activity. *Auxiliary* 2011;21:28–33.
- Wang Z, de la Fuente-Núñez C, Shen Y, Haapasalo M, Hancock REW: Treatment of oral multispecies biofilms by an anti-biofilm peptide. *PLoS One* 2015;10:e0132512.
- Yin W, Hu DY, Li X, Fan X, Zhang YP, Pretty IA, Mateo LR, Cummins D, Ellwood RP: The anti-caries efficacy of a dentifrice containing 1.5% arginine and 1,450 ppm fluoride as sodium monofluorophosphate assessed using quantitative light-induced fluorescence (QLF). *J Dent* 2013;41(suppl 2):22–28.
- Zasloff M: Antimicrobial peptides of multicellular organisms. *Nature* 2002;415:389–395.
- Zaura E, Nicu EA, Krom BP, Keijser BJJ: Acquiring and maintaining a normal oral microbiome: current perspective. *Front Cell Infect Microbiol* 2014;4:85.
- Zheng X, Cheng X, Wang L, Qiu W, Wang S, Zhou Y, Li M, Li Y, Cheng L, Li J, Zhou X, Xu X: Combinatorial effects of arginine and fluoride on oral bacteria. *J Dent Res* 2015;94:344–353.