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Caries prevention - An update for 2021

By Professor Laurence J. Walsh AO

This discussion on methods for dental caries prevention is based on published research from particularly the last 2 years that provides a platform for the current concepts of dental caries as a disease and thus the directions for caries prevention over the next decade and beyond. There is no doubt that we have come a long way in thinking about caries prevention. The goal now in each patient is to try to build a sustainable, healthy, oral biofilm that is diverse and compatible with oral health and conducive to the long-term stability of the dentition. This is a long way from the simple concept of eliminating all plaque twice daily as a non-specific approach. Instead, we recognise that biofilms are present 24/7 and thus we aim to cultivate an appropriate microbiome.[1,2]

The contribution of fungi

A key part of cultivating an appropriate microbiome is to realise that it's not just about bacteria - we must also consider fungi, as they also play an important role in dental caries. This has been recognised for over 10 years, through studies of children with severe presentations of caries. As a routine finding, they have very high levels of *Candida* species in their saliva and those levels usually correlate well with the levels of lactobacilli. This is no surprise because both like sugar-rich and acidic oral environments, so are often found together. *Candida* associate with mutans streptococci, and enhance their ability to form thick biofilms.

Different types of *Candida* species are involved in dental caries, not just *Canada albicans*, the most common species which around 50% of people harbour in their oral cavity. We now know that other *Candida* species are quite important, such as *Candida dubliniensis*. This species is detected very frequently in caries active children.[3] It is likely that many early studies probably missed this particular species because it gives identical results on some of the common specific tests for *C. albicans* such as the formation of germ tubes in the presence of serum.[4] An ecological approach to caries prevention must take these microorganisms on-board as well. They are not picked up in studies of bacteria that use 16 S molecular approaches, so more work is needed to explore what happens to the mycobiome (the fungi) across the early years of life, as that is an important missing piece of the puzzle of the oral microbiome.

A recent systematic review of the role of *Candida* species in caries in children, adolescents and adults shows that regardless of which age group is considered, high levels of *Candida* species in the saliva and oral mucosa are associated with a much higher caries prevalence, with up to a 2-fold elevation when the fungal load is high.[5] In any patient with a high fungal load, factors in their diet and lifestyle that elevate fungal load will also elevate their caries risk.

The tooth surface

We now recognise the importance of the enamel surface on which the biofilm is growing. In a recent large scale longitudinal study, we tracked dental caries and developmental defects of enamel (DDE) in a cohort from birth through to 6 years of age.[6] This study followed on from previous work in young children that we did, where DDE were found to be a major risk factor for caries.[7,8] In this most recent study of 725 children tracking over 14,000 primary teeth, it was shown that teeth with DDE had a much higher risk for caries and the risk elevation varied according to the nature of the surface change, which is to be expected. DDE with a pitted surface gave the greatest risk elevation (six-fold) versus those with just opacities (about 4.5 times).[6] The pitted areas provide protection for the biofilm. Hence, it is important that we check all our child patients for DDE, to identify those with physical defects of the surface, since such defects provide a protected physical niche for the biofilm to persist, despite brushing.

Beginning from birth

We need to think broadly about oral biofilms that contain both bacteria and fungi, how these biofilm microbial communities form and how we can build microbial communities from birth that are conducive to health.[1] We can no longer rely on everyone brushing with a fluoride toothpaste - as important as that is, as a preventive approach it's not enough in patients in high-risk populations. The ideal biofilm will be highly diverse in composition so that it can resist ecological pressures such as those from poor diets with high frequencies of exposure to sugar or to acid. Under such pressures, biofilm diversity narrows and the total number of species in the biofilm is less, as those that persist can survive in low pH conditions.

From birth, the mouth is in a highly dynamic situation and things can change quite dramatically. A microdroplet of saliva from an adult with high caries will inoculate the oral cavity of the infant with tens of thousands of cariogenic microorganisms. We first described this in the year 2000 as a major event in the initiation of dental caries in children.[7] Other lifestyle factors such as exposure to acidic drinks and sugar can have an incredibly powerful action and lead to dysbiosis - an imbalance in the biofilm composition - which narrows down the diversity of the microbiome and allows selective growth of acid tolerant microorganisms.[1]

Fostering friendly species

If our aim is a microbiome that sustains wellness, we need to think about what species are naturally present in the microbiome where they antagonise the target cariogenic microorganisms (both fungi as well as bacteria). Boosting such friendly species seems to be both logical and sustainable as an approach.

The early colonisers such as *Streptococcus sanguinis, S. gordonii* and *S. salivarius* are compatible with health, from the time of birth through the first year of life as the deciduous dentition begins to erupt and then into childhood, adolescence and beyond. With tooth eruption, as more hard surfaces appear in the mouth, we see the emergence of other sorts of microorganisms, particularly some of the Gram-negative organisms and the diversity increases further as the primary dentition comes to be fully erupted. The way that the oral flora develops strongly influences the risk of early childhood caries, as has been documented in case-control studies and in prospective cohort studies.[9-11]

The early colonisers such as *Streptococcus sanguinis* have important metabolic properties - not only do they readily colonise the surface of teeth, but they can also metabolise arginine and proteins to produce alkalis that elevate the pH of the plaque fluid. This prevents the emergence and dominance of acid tolerant organisms.

On the other hand, if the plaque biofilm becomes thick and the diet includes frequent exposure to sugar and acids, we expect to see the emergence of the keystone pathogen *S. mutans*. It influences the biofilm by laying down extracellular polymers as a thick matrix, which serves as a reservoir for substrate as well as a diffusion barrier, trapping acidic by-products from fermentation of carbohydrates in the plaque biofilm. As the thickness of the plaque increases, bacteria in the deeper, oxygen-depleted layers will alter their metabolism to allow them to survive under such conditions.[12]

Hence, as a strategy to control *S. mutans*, we want to encourage the growth of *S sanguinis*. We can live in harmony with a thin dental plaque biofilm that is composed mostly of such early colonisers.[13]

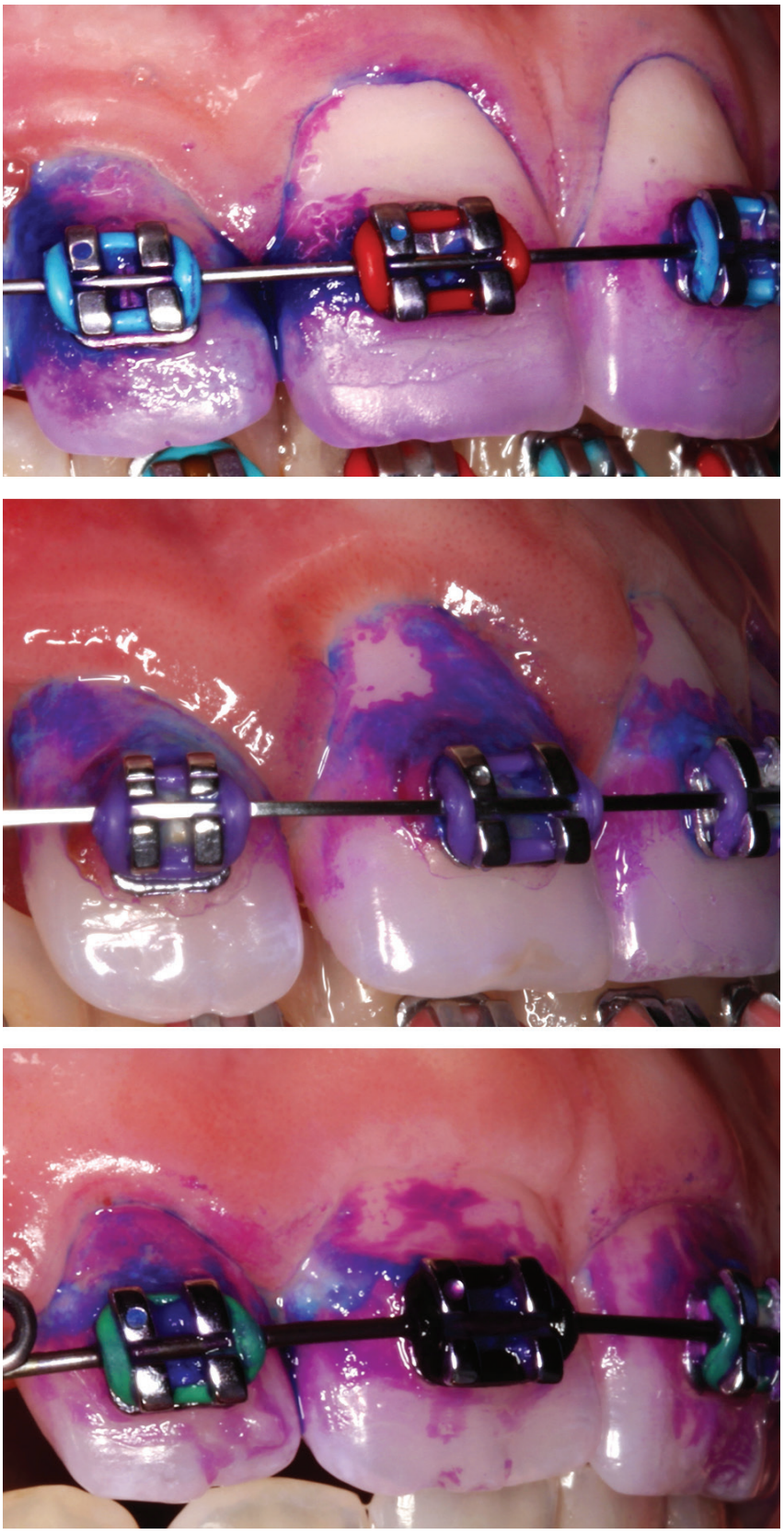

Figure 1. Three clinical examples of 3-colour disclosing gel (GC TriPlaque ID gel) used in orthodontic patients to show regional differences in the dental plaque biofilm.

Altering dental plaque biofilm ecology in young children

We need to cultivate or "re-program" the biofilm using agents that have prolonged actions, boosting the health-associated bacteria to keep the pathogens in check. An important theme in caries prevention is using milk-derived products such as casein phosphopeptides (CPP), which are the core of the technology in GC Tooth Mousse™ and related products containing CPP-ACP.[14,15] A recent study showed that GC Tooth Mousse can be used both as a dentifrice (toothpaste), even though it was not designed specifically for that purpose (as it lacks a surfactant).[16] The study compared plain (non-fluoride) Tooth Mousse used alone, in combination with a fluoride dentifrice, or the fluoride dentifrice alone, in a school-based toothbrushing study. All three groups of preschool children who were tracked over a six-month period completed their brushing at school under the supervision of specialists in paediatric dentistry. A strong ecological effect was seen from the CPP-ACP treatments, driving a large reduction in the levels of *S. mutans*. This finding extends what we had shown previously in 2-year clinical studies in Brisbane in infants who were using plain Tooth Mousse after tooth brushing with a child strength fluoride dentifrice.[17,18]

The teenage orthodontic patient

Another risk group are teenage patients having orthodontic treatment. We first discussed the issues with this patient group in the 1990s when we showed using saliva-based tests that levels of *S. mutans* increased by a massive amount (e.g. over 1000 fold) during the first 1-3 months after having bands and brackets placed.[19] The underlying principle is similar to the situation with DDE of the pitting type, as the orthodontic attachments provides protected locations for biofilm to form.[20,21]

As with young children, the concepts of orthodontic prevention have matured and changed over the years, moving beyond relying on mechanical plaque control alone to the concept of maintaining a healthy biofilm, i.e. the concept of wellness for oral microbiome. For several years now we have had the clinical tools to be able to see what the biofilm is doing.[12] In a 2019 paper on this topic, we illustrated the use of GC TriPlaque ID gel™ , a disclosing technology that shows biofilm thickness, maturity and acid production, for rapid use in the mouth as a type of visual Stephan curve.[21] If the biofilm is thin and healthy because the patient has a good standard of oral hygiene, it will stain pink. Such areas of biofilm will be less than 24 hours old. In such situations, the patient will not develop caries around their orthodontic attachments. On the other hand, biofilm which is stained in a purple colour is thick and mature and can readily become cariogenic. Biofilm that stains a light blue colour is already producing acids at a high level, so caries is already occurring and a white spot lesion may be forming on the enamel beneath the biofilm. Here, the biofilm has become less diverse and acid production is the norm, rather than alkali production (Figures 1 and 2).

Altering dental plaque biofilm ecology in orthodontic patients

When we studied what CPP ACP does to the virulence of biofilms that were single species *(S. mutans)*, dual species *(S. mutans* and *Candida albicans)* and polymicrobial in nature (over 300 species), we found that it lowers the volume of the biofilm, impairs the adhesion of biofilm to substrates and reduces acid production. Moreover, the composition of the biofilm changes, with more health-associated bacteria and less cariogenic bacteria.[22] The increase in *S. salivarius* and *S. sanguinis* is linked to the biofilm changes, since both of these use the arginine deaminase system (ADS), which generates alkalis. We described these effects as being prebiotic, not only because CPP are ingestible, but also because the proteins of the CPP are metabolised eventually to alkali.

A similar prebiotic effect for CPP-ACP on biofilms was shown in recent research from the University of Melbourne, where CPP-ACP was used in conjunction with stannous fluoride twice daily.[23] They found that enamel demineralisation reduced by 50% and there was change in the balance of the microflora, in the same pattern as we had seen, with more ADS "healthy" bacteria and fewer pathogens. The CPP-ACP stabilises the stannous fluoride and with it forms nano-filaments that bind the CPP-ACP nanocomplexes onto the tooth surface, giving a sustained effect both as a remineralising agent and as a prebiotic agent.[24]

Phytochemical prebiotics

My lab has had a long-standing interest in prebiotics from plants and much of our work has been on cranberry-derived compounds and especially A-linked pro-anthocyanidins (A-PACs).[25,26] That such molecules exist is not surprising given that plants must have ways to protect themselves from predation - including from microorganisms. Many plant-derived compounds have broad spectrum antimicrobial actions, which is not what we were looking for - rather, we were searching for a prebiotic that could reduce the virulence of the biofilm and, as discussed above for CPP-ACP, drive a beneficial change in the biofilm composition, restoring it to a "healthy" profile. We showed that cranberry A-PACs can suppress acid production, slow down the rate of biofilm growth and also disrupt the biofilm's structure. They do not kill bacteria, so resistance will not develop. They inhibit the action of the glucosyltransferase enzyme from S. mutans, which is the key enzyme for building a dense glutinous plaque matrix.

When we studied the effect of a cranberry A-PAC on biofilms that were single species (*S. mutans*), dual species (*S. mutans* and *Candida albicans*), or polymicrobial in nature, we found that it lowered the volume of the biofilm, impaired the adhesion of biofilm to substrates and reduced acid production. Moreover, in a similar pattern to what we had seen with CPP-ACP, we found that the composition of the polymicrobial biofilm changed, with more health-associated bacteria which used the ADS and fewer cariogenic bacteria. Levels of *S. sanguinis* rose by over 90-fold.[27,28]

We went on to do a phase III randomised controlled clinical trial in teenage orthodontic patients to test our prebiotic concepts, delivering these in a fluoride dentifrice with 10% CPP-ACP (the same level as in Tooth Mousse), or CPP-ACP plus the cranberry A-PAC prebiotic agent, with the same fluoride dentifrice vehicle as a control. As expected, the CPP-ACP alone had strong prebiotic effects, while the CPP-ACP plus A-PAC showed even stronger effects. Both boosted the levels

of health-associated organisms like *S. sanguinis* and suppressed the keystone pathogen *S. mutans*.[29] To us, this seems a practical way of leveraging a common delivery system to achieve worthwhile outcomes around a healthy microbiome.

Arginine

As a key substrate for the ADS system, it is logical to think about how much benefit can be gained by using arginine in its various chemical forms directly. Exposing biofilms to arginine does start to alter their behaviour and composition, so there is a potential role for adding it to the repertoire of preventive prebiotic agents. Recent reviews including systematic reviews and meta-analyses have concluded that there is some low-grade evidence that arginine in a dentifrice (including in commercial products) may be beneficial, but they rated the studies as having a high risk of bias.[30] Thus, much more work is needed to reach a firm conclusion, even though the underlying science around arginine being a prebiotic is solid.

Probiotics

These products contain living bacteria and may be formulated as a gelatin capsule that dissolves in the gut, thereby releasing the bacteria, or the bacteria may be incorporated into milk or milk-based products. Probiotics are typically bifidobacteria or lactobacilli of different sorts, of benefit to the gut flora. Such bacteria may not be found normally in the mouth, where the conditions are vastly different. This poses some challenges - including whether such organisms can "take hold" and become part of the oral microbiome and whether their inherent properties of acid tolerance and acid production make them possibly more damaging than beneficial. Some recent studies have shown that certain probiotics delivered through yoghurt or milk can lower salivary levels of *S. mutans*, but the effects seem transient.[31-33] Data from the milk only controls suggest that the effect may be more a reflection of the milk vehicle components than of the bacteria added to the milk. Some work has shown benefits from using reconstituted powdered milk with *Lactobacillus paracasei* when such probiotic milk was consumed daily. There was an increase in the likelihood of white spot lesions regressing back to sound enamel, which could again reflect the casein components in the milk.

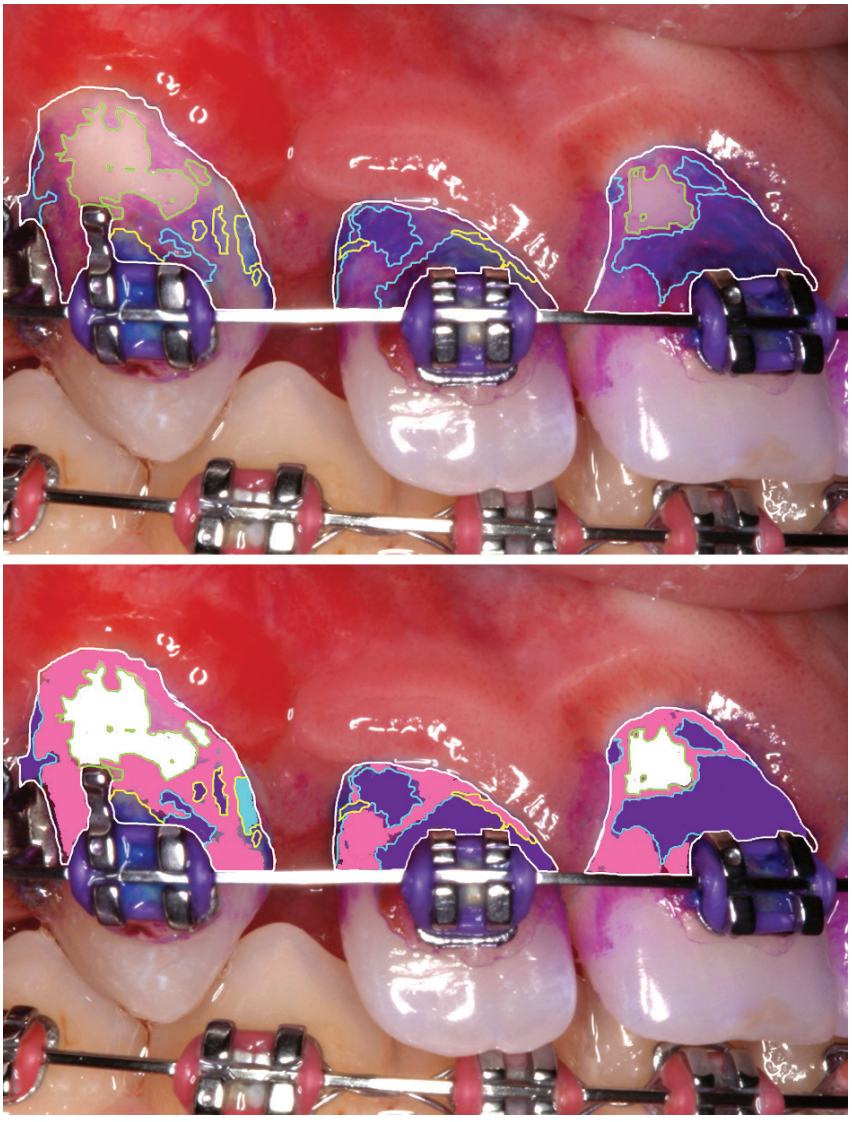

Figure 2. A matched pair of images showing zones of 3-colour disclosed dental plaque above the archwire outlined (upper panel) and then classified into three types on the lower panel: pink (thin), purple (thick/mature) and blue (acid producing). White indicates clean areas with no stained biofilm present. Such large areas of mature thick biofilm can readily progress to be acid producing when the oral environment has prolonged periods of acidic pH conditions. Already one area of biofilm on the cervical aspect of the canine tooth has become highly acid producing.

Several systematic reviews of the dental probiotic literature have been published recently.[31,34,35] The results are mixed and the only consistent benefits are seen in those products that are based on milk. It has been known for the past decade from studies using labelled bacteria that probiotic bacteria designed for boosting gut health do not bind to dental hard tissues, nor do they integrate into oral biofilms.[36] Hence, effects will be mostly through the salivary part of the microbiome and will be transient rather than long lasting. Such a conclusion is supported by a recent systematic review of the use of probiotics in patients having fixed orthodontic appliance therapy.[37] Probiotics did not give a great or persisting benefit. That suggests that this idea may not be going very much farther.

Synbiotics

A synbiotic is the combination of a probiotic with a prebiotic. The thought process is that such a combination

approach might compensate for some of the limitations or drawbacks of using just one of these approaches. The synbiotic concept is still in its very early days.[38] In the lab when used to treat a mixture of bacteria, a synbiotic can enhance ADS activity, as one would expect.[39] It is likely that the probiotic aspect will re-imagined, changing from a gut lactobacillus to an oral bacteria that is known to make bacteriocins - compounds that antagonise or suppress the pathogens of interest. This could help address the "Achille's heal" problem of lack of binding and integration of the probiotic into the oral microbiome.

Xylitol

Xylitol has been studied since the early 1980s. It can be made synthetically from natural products, or extracted from natural sources, such as the Birchwood tree. In the lab, it can alter *S. mutans* binding and metabolism, but those effects decline as bacteria adapt to it. It can interact with calcium ions and that might boost remineralisation. Recent interest in xylitol has been sparked by studies of its possible benefits in patients with conditions such as diabetes, obesity and osteoporosis.[40] Such changes may occur through what xylitol does to the gut microbiota. As this story develops, it may well be that its benefits for general health (other than being a sugar substitute) gain greater attention. It is still used in many dental products, including chewing gums and Tooth Mousse, as well as some dentifrices. The doses ingested from using such products are small, which is why laxative effects are uncommon - but do occur when large amounts are ingested.

Other phytochemicals

Many plant extracts have been tested for possible caries preventive actions.[25,41] Common topics for research include extracts from liquorice or cinnamon, which have broad spectrum antimicrobial actions because of their polyphenol content. Using broad spectrum agents does not seem sensible when one can achieve the desired result using prebiotics in a more subtle way, with no risk of resistance developing. Gallates from tea seem quite promising, as these can lower biofilm virulence by inhibiting glucosyltransferase and there is a solid body of clinical evidence for modest anticariogenic actions.[42]

In contrast, the evidence base for many other plant-derived compounds (including herbs) is small, with little good quality clinical research. Much research has been done in the laboratory using simple one species culture plates rather than biofilms or more complex models. Having said this, antimicrobial effects are often broad spectrum and effectiveness is poor. They do not show desirable microbiome modifying actions. Thus, despite being popular, Echinacea and aloe vera do not fare well when compared to existing well-known caries prevention agents,[43,44] or to the prebiotics we have discussed.

Any plant extract needs to be pure, have a consistent chemical composition and a well-established safety profile, as well as acceptable taste when included into dental products. Some very potent agents (e.g. cinnamaldehyde) have a pleasant odour but a very unfavourable taste.[45] This problem does not occur with A-PACs or CPP.[29]

Antimicrobial peptides

AMPs can be natural (such as those made by bacteria or algae), or they can be synthetic. Over 40 synthetic AMPs have been tested as possible caries preventive agents. Some impair the growth of *S. mutans* as well as fungi and around 10 are known to bind to hydroxyapatite and promote re-mineralisation. Most studies of AMPs are lab-based using simple models.[46] To date, no studies have explored whether AMPs can alter the composition of the dental plaque biofilm. It seems a long journey before AMPs will reach clinical trials. Issues such as their poor stability need to be addressed.

Quorum sensing

The final new direction in caries prevention is modifying quorum sensing systems in biofilms, to block the normal communications between cells and confuse the signals that are being given. The analogy is like attacking telephone towers and landlines, or sabotaging the Postal Service, so that false messages are sent and false information is received about the outside world. The concept is still relatively new.[47] Of interest, when CPP are used, they elevate calcium ion levels and fluoride ion levels inside the biofilm, which suppress acid production.[14] There is some interest in taking quorum sensing molecules and applying those directly, but challenges around stability and penetration will need to be resolved.

Conclusions

There are several new and exciting directions for caries prevention today. The modern thought process revolves around trying to build and then sustain healthy biofilms in the oral cavity for life. We now know that we can do that now using prebiotic approaches. These actions add to, rather than replace, lifestyle changes and oral hygiene. Our aim is to build a healthy biofilm through the appropriate diet and shape this with the use of prebiotics or even synbiotics, particularly in the early years of life, to ensure a resilient diverse biofilm. While historically CPP ACP began as a re-mineralising agent, we now understand its effects on bacteria, on plaque acid production and on plaque composition. It may well be that some of its most impressive and important long-term actions are through its prebiotic effects.

The agents discussed herein are organic in nature and come from nature. The lessons from nature are important. There are already some natural control systems at work - so how can we empower those? That broad question is now being asked. The same question has already been asked when it comes to the gut flora.[48] The concept of preserving a healthy gut microbiome is central to much of modern medicine and there is growing evidence that gut microbiome dysbiosis or imbalance is linked to a range of conditions including colorectal cancer and malabsorptive conditions of various types.

Unlike the gut flora, the oral flora is easy to sample and assess. We have the clinical tools (like TriPlaque ID gel) to do that. We have agents (like CPP-ACP) that can help change the microbiome back to a healthy profile. We already understand what a poorly managed lifestyle does to the microbiome. Hence, it's putting those pieces of the story together so that our patients can understand. They already have likely encountered the gut microbiome story, so its extension to the start of the GI tract should come as no surprise. We can assess it and we and the patient can change it. This is a word of empowerment for clinicians to now do exactly that.

About the author

Emeritus Professor Laurence J. Walsh AO is a specialist in special needs dentistry who is based in Brisbane, where he served for 36 years on the academic staff of the University of Queensland School of Dentistry, including 21 years as Professor of Dental Science and 10 years as the Head of School. Since retiring in December 2020, Laurie has remained active in hands-on bench research work, as well as in supervising over 15 research students at UQ who work in advanced technologies and biomaterials and in clinical microbiology. Laurie has served as Chief Examiner in Microbiology for the RACDS for 21 years and as the Editor of the ADA Infection Control Guidelines for 12 years. His published research work includes over 330 journal papers, with a citation count of over 15,400 citations in the literature. Laurie holds patents in 7 families of dental technologies. He is currently ranked in the top 0.25% of world scientists. Laurie was made an Officer of the Order of Australia in January 2018 and a life member of ADAQ in 2020 in recognition of his contributions to dentistry.

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