# Dental plaque fermentation and its role in caries risk assessment

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**Key words: Dental plaque, fermentation, cariology**
**Short title: Dental plaque fermentation**

**Abstract**
In contemporary dental practice, the role of dental plaque fermentation in the dental caries disease process is well understood, but difficult to assay for and to demonstrate to patients as an educational and motivational tool. This paper provides an overview of the current concepts of dental plaque fermentation with reference to the health/unhealthy biofilm concept of dental plaque. It explains the basis for chairside tests for plaque fermentation, and identifies measures which can be targeted to address production of harmful organic acids by dental plaque.

## Introduction

Dental caries is initiated by the process of fermentation, in which the production of strong organic acids such as lactate, formate and pyruvate cause demineralization of the tooth surface. Stephan in his classic studies in the early 1940's showed that dental plaque exposed to sucrose could rapidly produce acids, causing a rapid drop in pH followed by a gradual recovery toward the baseline plaque pH[1,2]. Since that time, a causal association between the production of strong acids from plaque in response to sucrose and caries activity has become well established.

Some plaque bacteria can produce only the pH fall of the Stephan curve, whereas others (arginolytics) can produce both the fall and the rise – the latter through degradation of nitrogenous compounds, such as the peptide sialin and the amino acid arginine, the end-products of which can raise plaque pH[3,4]. The balance between these different metabolic outcomes of bacterial activity dictates the shape of the Stephan pH curve. In fact, analysis of plaque fluid samples taken at intervals during the Stephan pH curve following a sucrose mouth rinse has revealed that levels of lactate rise after the rinse, then fall during the pH recovery phase. In contrast, levels of acetate and propionate fall after sucrose rinsing, then rise again[5]. As will be discussed further below, this latter group of weak organic acids play a vital role in buffering pH changes in plaque.

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## Fermentation and its effects

Many dental plaque bacteria can ferment carbohydrate substrates, and a large number of organic acids (of varying potency for demineralization) result from this process. For this reason, it is logical to look at the plaque biomass and the net result of fermentation, rather than to focus narrowly on just one species or just one organic acid.

An assessment of acid production from carbohydrate by dental plaque bacteria can be used to assess the cariogenicity of dental plaque from a particular site. As fermentation proceeds, the plaque pH decreases to approximately 4 within 5 minutes, and this state of lowered pH persists for up to several hours, depending on the presence of salivary protection factors. It is now well recognized that acid production following a sucrose challenge differs both between patients and between sites in the same patient.

Acid production within plaque affects the nature and composition of the dental plaque microflora. Bacteria with a high tolerance for acid (aciduric bacteria) which can also produce large amounts of acid can selectively overgrow within the microflora of supragingival plaque. This includes organisms within the mutans streptococci grouping as well as lactobacilli. In fact, the numerical emergence of mutans streptococci in dental plaque is often preceded by other types of aciduric bacteria. Many streptococci are relatively acid tolerant, while highly aciduric bacterial species are few in number (Table 1).

When considering the role of supragingival dental plaque in dental caries, the proportion of Gram-positive facultative acid-producing bacteria (particularly mutans streptococci and lactobacilli), has direct relevance to the pathogenicity of the plaque. These microorganisms tolerate a low pH environment, and thrive when the diet is high in cariogenic substrates such as table sugar (sucrose). *Streptococcus mutans* and *Streptococcus sobrinus* produce insoluble extra-cellular

| Table 1. Examples of acid-tolerant oral bacteria | |
|---|---|
| **Acid tolerant @ pH = 4** | **Acid tolerant @ pH = 5** |
| *Streptococcus mutans*<br>*Streptococcus sobrinus*<br>*Lactobacillus spp.*<br>*Actinomyces odontolyticus*<br>*Enterococcus faecalis* | *Streptococcus sanguis*<br>*Streptococcus oralis*<br>*Streptococcus gordonii*<br>*Streptococcus anginosus*<br>*Streptococcus constellatus*<br>*Streptococcus intermedius*<br>*Streptococcus mitis*<br>*Streptococcus salivarius*<br>*Streptococcus vertibularis*<br>*Actinomyces viscosus* |
| (based on data [79,14,80]) | |

polysaccharides from sugars, both as a means of forming a dense protective biofilm, and as a means for storing surplus substrate. Strains of mutans streptococci vary in terms of their ability to synthesize water-insoluble glucans. These polymers play an important role in initial caries development by increasing the adherence of mutans streptococci and their accumulation within dental plaque, particularly in young children[6,7].

There is an important influence of the resting pH of saliva on the microbial ecology of dental plaque. Within the plaque environment itself, the resting pH results from a delicate balance between alkali and acid generation, which is in turn dependent both on the bacterial composition of the plaque and on the supply of substrates and buffers from, and metabolite clearance into, flowing oral fluid[8]. Because of this, the resting plaque pH varies regionally in the oral cavity because of site-specific effects of saliva. It is generally lowest in interproximal regions, which lack access to saliva once the plaque biofilm has become sufficiently thick to occlude the gingival embrasure beneath the contact points.

After a sucrose challenge, plaque acid production is greater for patients with high levels of mutans streptococci (greater than 1 million per mL) in their saliva and a greater proportion of mutans streptococci in dental plaque than in patients with low levels in either saliva or plaque. For this reason, direct measurement of plaque acid production *ex vivo* provides a surrogate measure of cariogenic fermentative bacteria, without discriminating between the particular species which may be present.

## The balance of organic acids

In the process of fermentation by dental plaque, lactate is a major product from the fermentation process, particularly when sucrose is present in large quantities. Other acid by-products of glucose metabolism by the mutans streptococci include acetate, formate, and pyruvate. Within carious dentine, additional acids derived from fermentation by bacteria include propionate, butyrate, succinate, valerate, and caproate. Depending on the supply of nutrients, *Streptococcus mutans* can alter its patterns of acid production via the glycolytic pathway. For example, when sucrose is present in small amounts, the major metabolic products of the glucose and fructose derived from this will be pyruvate, acetate and formate, while a situation of excess results in the production of mostly lactate with a lesser amount of pyruvate.

Because of the variety of organic acids, it is important to consider the effect of different concentrations of these. For example, dental plaque which has formed in a low cariogenic environment and has limited fermentation capabilities will produce primarily **acetate**, (with lesser quantities of **propionate** and **butyrate**), weaker acids which can effectively buffer plaque pH changes[9,10]. In contrast, plaque which has formed in a highly cariogenic environment produces large quantities of **lactate, formate** and **pyruvate**, stronger organic acids that can more readily demineralize dental enamel [11-13] (Table 2).

## The rationale behind assessing overall acid production

Acid production at low pH is an important trait of cariogenic bacteria. For this reason, an assessment of acid production by

| Table 2. Selected acids and their acid dissociation constants ($K_a$) |
|---|
| Destructive inorganic acids (etching action)<br>Phosphoric acid (710)<br>Hydrofluoric acid (67)<br><br>Destructive organic acids (demineralizing action)<br>Lactate (14.0)<br>Formate (17.7)<br>Pyruvate (320)<br><br>Protective organic acids (buffering action)<br>Acetate (1.75)<br>Propionate (1.62)<br>Butyrate (1.52)<br>Carbonic (0.044) |
| Acid dissociation constants are given in units of $10^{-5}$ X $K_a$ in mol/dm3. Higher $K_a$ values indicate stronger acids. (Based on data [81, 82]) |


***Figure 1: The Plaque-Check+pH kit for testing plaque fermentation (GC Corporation, Japan) uses a colourimetric method to demonstrate the pH drop of the Stephan curve when intact plaque is challenged with sucrose.***

dental plaque bacteria can be a useful addition to the process of caries risk assessment. There is considerable clinical evidence that cariogenic conditions are associated with increased proportions of microorganisms capable of acid production at a low pH. With a highly cariogenic diet, shifts occur in the dental plaque microflora, with increased numbers of specific organisms, including mutans streptococci, lactobacilli, and strains of *Bifidobacterium* and *Actinomyces* species, in the case of root surface lesions[14].

Numerous clinical studies have established that the proportions of microorganisms designated as capable of acid production at low pH conditions, are significantly increased in plaque from patients with high caries risk[15-20]. Direct comparative studies of dental plaque acid production in caries-resistant vs. caries-susceptible adult patients have shown the usefulness of this approach. For example, both the amount and rate of production of lactate are lower in caries resistant patients, while the level of acetate is higher both before and after exposure to sucrose [9]. Caries-free subjects (based on past experience) tend to show a higher plaque pH after a sucrose challenge. Having said this, it needs to be remembered that in an individual patient, the frequency of acidogenic episodes in their lifestyle will be more important in caries progression than the degree of acidogenicity during any one episode measured *ex vivo* (at chairside) [21].

## Methods to assess acid production

A number of tests have been developed to detect the presence of acidogenic bacteria in dental plaque, using specific organic acids such as lactate. The Clinpro Cario-L-Pop test from 3M-Espe employs a biochemical method to assess lactic acid using biofilm samples from the tongue. It utilizes the enzymatic oxidation of lactic acid to pyruvate by lactate-dehydrogenase coupled to a cascade of redox indicators to generate a purple-blue colour in 2 minutes, which is then scored against nine fields on a colour chart.

Colourimetric tests for chairside use provide a simple means for assessing the pH-lowering potential of dental plaque, without focussing on any one particular organic acid (Figs. 1 and 2). This method is based on the pH drop which occurs in plaque samples when exposed to an excess supply of sucrose, an effect which has been described in the literature numerous times in the past 30 years. The addition of sucrose to a cariogenic dental plaque markedly lowers the pH, with the lowest value at 5 minutes, after which it slowly rises[22]. Employing pH indicator dyes to measure dental plaque pH is simpler than using pH microelectrodes, and thus is preferred as a clinical technique. Using sucrose-exposed non-dispersed plaque (intact on a sampling device) gives more lactate than sucrose-exposed dispersed samples of plaque[23], and thus provides a closer parallel to the plaque biofilm configuration *in vivo*. Assessing global plaque pH rather than lactate levels *per se* addresses the potential for effects from five or more other acids, and thus may be a more sensitive means to evaluate the cariogenicity of the plaque.

## Regional variations in the oral cavity

Caries risk status is linked causally with increasing plaque levels of highly-acid-tolerant, acidogenic bacteria and an increasing plaque-pH-lowering potential[19]. Thus, after a sucrose challenge, pH values in dental plaque *ex vivo* will be lower in caries-active patients or sites, compared with caries-free sites or patients. Nevertheless, when selecting sites for sampling supragingival plaque, it must be remembered that plaque pH varies from site to site in the mouth, however there is a consistent pattern of site distribution because of the extent of contact with saliva, which can clear substrates and buffer plaque acids[24]. Clearance of substrates by saliva is slowest in the anterior region of the maxilla, and in the canine/premolar region of the mandible. For this reason, sites in the anterior maxilla, such as the labial cervical aspect of the maxillary incisor and canine teeth (Fig. 3), consistently give lower pH (by 0.5-1.0 pH units) than similar sites in the mandible, as well as lower plaque fluoride levels.[16, 25] The rate of clearance of acids from plaque into the overlying salivary film is greatly retarded at low salivary film velocities. Because of this, plaque located in regions of the mouth with a low salivary film velocity will achieve pH values lower than those of plaque of identical dimensions and microbial composition located in areas where salivary film velocity is high [26]. This explains why caries occurs preferentially in sites in the dentition characterized by a

relatively high exposure to carbohydrate and diminished salivary clearance and buffering effects [27,28].

Similarly, the minimum pH in plaque at approximal sites is lower than in plaque from buccal surfaces, and remains below resting levels for up to 120 minutes after sucrose challenge [29]. Nevertheless, plaque from sites of active caries (either white spot lesions or frank cavitations) will show a greater pH fall after sucrose challenge, and a slower recovery than plaque from sites without active caries [30, 31,19]. As well as site-by-site analysis, evaluation of pooled dental plaque samples for acid production is also possible. Pooled samples of plaque obtained from individual patients with clear differences in caries experience would be expected to show differences which align with caries susceptibility [32].

## Clinical technique

Sites for sampling should include those sites most at risk for development of dental caries. As noted above, plaque varies regionally in the oral cavity because of site-specific effects of saliva. It is generally more fermentative in regions which lack access to the protective effects of saliva, e.g. cervical surfaces of maxillary incisor teeth, mandibular canines and premolars, and inter-proximal sites. Plaque samples can also be taken from sites with white spot lesions or frank cavitations. Plaque from sites of active caries will show a greater pH fall after sucrose challenge than plaque from sites without active caries.

Aging of dental plaque (particularly if undisturbed for up to 2 days) gives a greater level of acid production than more immature plaque. Thus, sites with "old" plaque should be selected for assessment. It is only necessary to collect plaque from one side of the mouth only as the fermentation ability does not vary substantially from one side to the other.

A commercial kit to assess plaque fermentation has been developed (Figures 1-4), which includes disposable plaque collection instruments. Immediately prior to collecting the plaque sample, an air syringe is used to lightly dry the site to be sampled, to reduce the risk of contamination with saliva (which may cause an inaccurate result). The plaque sample is then dipped for 1 second into an indicator solution (which contains sucrose and pH indicators), and then taken out and observed after 5 minutes. In the Plaque–Check+pH kit, the commencing colour of the solution (green) changes as acids are produced, with the final plaque pH measured by checking the colour against a chart (Fig. 2). In patients with a low caries

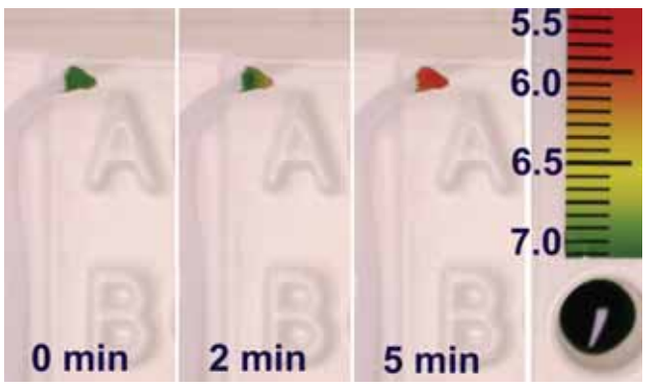


***Figure 2: Time lapse images showing changes in pH over time as fermentation occurs on a plaque sample (bound onto the end of the plaque collecting instrument). A pH scale has been superimposed on the colour comparison chart***

risk, there will be limited fermentation and buffering by weak organic acids will limit pH changes in the plaque. A green colour after 5 minutes indicates a normal pH around 7.2, indicating that the plaque has a low fermentation ability and the pH has been unaffected by the sucrose challenge. A yellow or orange colour indicates a final pH of 6.0-6.6, while a pink or red colour indicates a final pH of 5.0-5.8. Clearly, for samples which score in the yellow or red region, preventive action is recommended. This should include discussion with the patient about the dangers of plaque acid production which can lead to prolonged demineralization and possible cavitation from dental caries. Repeating the test can be used to assess compliance with advice regarding changes in diet and lifestyle.

## Targeting acid production as part of a caries prevention strategy

*Preventive strategies which are based on the concept of limiting fermentation by dental plaque include the following [33].*

Dietary restriction of sucrose and other fermentable simple sugars between meals. The classic Vipeholm study in the 1950's demonstrated that frequent intake of foods with high sucrose concentrations increases caries activity, and the primary mechanism for this is lactate production by cariogenic bacteria rather than acetate, propionate, or butyrate [33].

Dietary replacement of sucrose by poorly fermentable or non-fermentable materials such as maltose, xylitol, sorbitol, sucralose, trehalose, and isomalt. In this context, it must be remembered that there is no significant difference in the acidogenic potential between sucrose and extrinsic sugars derived from fruits[35,36]. In fact, virtually all foods which contain carbohydrates cause the pH of plaque to fall below 5.5[37].

Dietary restriction of high starch foods between meals. Particles of high-starch snack foods such as potato chips, doughnuts, and salted crackers have been shown to remain longer on the teeth than those of high-sucrose, low-starch foods, due to slower clearance. During retention on the teeth

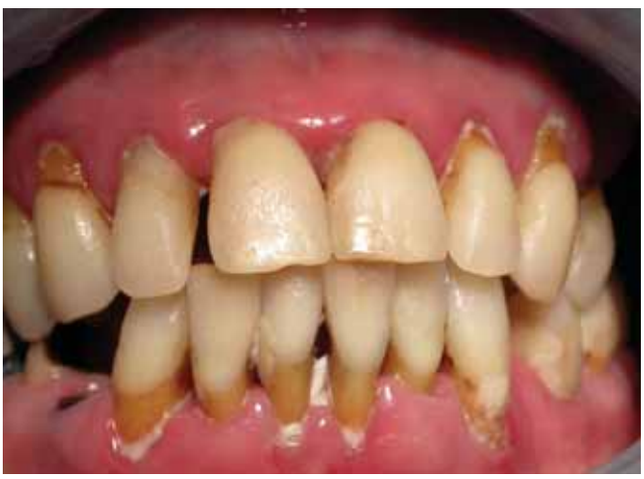

***Figure 3: Clinical example of a 55 year old patient who in the past year had noticed a sudden increase in caries incidence. Deposits of plaque can be seen on the cervical aspect of most teeth.***

for up to 20 minutes, breakdown of starch by salivary amylase releases a number of fermentable simple sugars, giving total levels of fermentable sugars similar to high-sucrose confectionery products. Fermentation of the accumulated sugars occurs in retained food particles, releasing a range of organic acids [38, 39]. Moreover, some bacteria, such as *Actinomyces viscosus,* can utilize starch for energy without the requirement for amylase. For these reasons, high starch snack foods are an important component of the caries risk profile.

Dietary restriction of highly acidic foods and drinks, which encourage the development of an aciduric plaque microbiota. This includes soft drinks, energy drinks, and fruit juices. Many of these (including fruit drinks designed for infants) have a low pH and a high titratable acidity. Clinical studies have revealed that some drinks depressed the plaque pH to below 5.5 within 5 minutes of drinking and were as cariogenic as a 10% sucrose solution [40].

Use of sorbitol- or xylitol-containing chewing gums, which reduce the acidogenic potential of dental plaque and neutralize lactate produced by dental plaque [41-44]. Gum-chewing also stimulates a protective salivary flow when used after an acidogenic stimulus, and may enhance salivary function, especially in subjects with low flow rates [45]. Sorbitol and xylitol gums can promote enamel remineralisation, but are less effective than gums containing casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) [46].

Use of milk-based foods, such as cheese, as snacks, since these which can prevent or reduce acid production. Dairy products, including cheese and milk, can reduce the cariogenicity of fermentable substrates, and this has been demonstrated in a variety of animal and *in vitro* systems. Clinical studies have shown that processed cheese is hypo-acidogenic, anti-acidogenic, and prevents demineralization as well as enhancing remineralization [47, 48].

Application of products containing Recaldent ® (CPP-ACP), such as GC Tooth Mousse. These work by several mechanisms. First, CPP-ACP binds well to plaque, providing a large calcium reservoir within the plaque, which slows diffusion of free calcium and reduces the ability of plaque fluid to dissolve the underlying enamel when the pH falls during sugar exposure [49]. The therapeutic importance of elevating dental plaque calcium concentrations has been demonstrated by several research studies [55, 51]. Second, CPP-ACP provides a source of calcium for remineralization [52]. Third, CPP-ACP also maintains a state of super saturation of phosphate ions with respect to tooth enamel. These phosphate ions can help buffer plaque pH. Finally, CPP-ACP inhibits the growth of *Streptococcus mutans* and other odonto-pathogens. This may be due in part to the increased pool of calcium ions in dental plaque [53].

Twice daily toothbrushing, to reduce the thickness of the dental plaque biofilm. Aging of dental plaque (particularly if undisturbed for up to 2 days) gives a greater level of acid production than more immature plaque [54]. As dental plaque becomes mature, the non-mutans streptococci with high acidogenicity and acid-tolerance will establish an acidic environment. This environmental shift permits more acidogenic and acid-tolerant bacteria such as mutans streptococci and lactobacilli to enter the dental plaque ecosystem [55,56].

Use of high fluoride toothpastes, since fluoride impairs energy utilization and thus acid production via the fermentation process, because of its effects on the two enzymes enolase and $H^+$/ATPase[57-60]. High levels of fluoride (0.16-0.3 mol/L) will kill bacteria, and these may be achieved through topically applied professional products such as gels and varnishes [58].

Use of fluoride-containing toothpastes with sodium bicarbonate, which result in significantly less plaque acid formation after a sucrose challenge than a conventional (adult-strength) fluoride toothpaste [61].

Rinsing with sodium bicarbonate solution, to both buffer plaque acids and reduce the aciduric environment. Buffering effects from saliva or mouthrinses may add to the buffer systems already present in plaque, such as soluble proteins, peptides, organic acids, and phosphate [62].

## Dietary advice

Limitation of sucrose intake between meals is an important lifestyle change to promote in patients with high caries activity. The extent of acid production by dental plaque can be monitored over time using chairside plaque acid tests, and the information from this used for reinforcement and feedback at recall visits. Sucrose is the most cariogenic substrate. The high cariogenicity of dental plaque formed in the presence of

sucrose can be explained by the high concentration of insoluble glucan polymer material in its "sticky" matrix[63].

The acidogenic properties of dental plaque reflect both the dietary habits and the caries status of the patient. Thus, a high level of dental plaque acid production indicates that the diet and lifestyle is conducive to the growth of aciduric, acidogenic microorganisms. It may be high in fermentable substrates, acids (particularly from fruit juices, softdrinks, and energy drinks), and caffeine. Because of the conditioning effects of these influences on the ecology of dental plaque, greater acid production occurs in patients who have had a previously high-sugar diet than in those with low-sugar diets, when the same foods are ingested by both[64].

Patients may require education on the types and amounts of "sugar" which are found in various types of foods. Sucrose is commonly used in processed foods, drinks, and medicines as a flavour enhancing agent. The levels can be surprisingly high, for example softdrinks typically contain 12-14% sugar, thus 1 can (375 mL) of softdrink contains 45-50 grams of sucrose, which is equivalent to 11-13 teaspoons. High levels of sucrose are typically found in foods that have little nutritional value in terms of fibre, vitamins or minerals, and these are termed "empty calories". Excessive sucrose consumption can lead to weight gain, obesity, and non-insulin dependant (Type 2) diabetes mellitus. As argued by Newbrun, better labelling of foods and drinks which disclosed the actual concentration (percentage by weight or volume) of sucrose and other sugars would help consumers in choosing products which would be less likely to contribute to dental caries[65].

"Natural" sugars found in fruits can contribute significantly to plaque acid production, as can starchy products such as sweetened and unsweetened breads, and potato chips. This is because of the breakdown of starches within the salivary environment by alpha amylase and other enzymes. As would be expected, greater levels of starch hydrolysis cause greater plaque acid production[66,67]. Regardless of the source of fermentable substrate, in the caries-prone patient, a more rapid decrease in plaque pH occurs when exposed to substrates (from the production of lactate, formate, and pyruvate) than in a caries-free patient, resulting in a lower final plaque pH[68]. The duration of the drop in plaque pH can be considerable. For example, the period when the plaque pH is below 5.5 is 75 minutes after contact with sucrose in solution (such as a softdrink), and 140 minutes after eating a roll with jam and then drinking sweetened coffee[69].

A further issue to be considered is the use of sweeteners by caries-prone individuals to reduce their intake of sucrose and other fermentable sugars. Such substitution deprives dental plaque bacteria of the ability to use these as an energy source in the process of fermentation[70]. As noted above, a number of sweeteners now exist which are effective replacements for dietary sucrose. Materials such as sucralose, leucrose, trehalose, palatinose and isomalt (which are chemically similar to sucrose) are now being used in confectionery. A potential concern with some of these "engineered" substitutes (i.e. chemically altered forms of sucrose) is that they may undergo extracellular cleavage by certain autochthonous micro-organisms such as *Stomatococcus mucilaginosus*. The resulting cleavage products, such as glucose and fructose, could then be used by cariogenic bacteria[71]. This emphasizes the importance of clinical trails with microbiological and clinical parameters when assessing the value of replacement sweeteners.

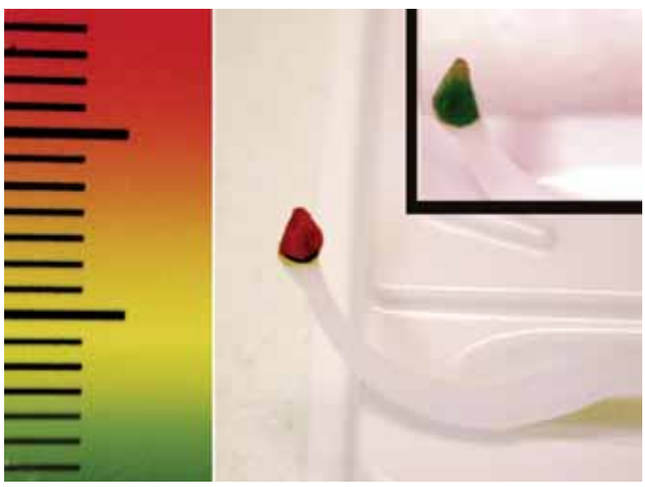

***Figure 4: A plaque sample taken from the labial aspect of the left maxillary canine tooth (tooth 23) shows a pH drop to less than 5 at 5 minutes when challenged with sucrose, demonstrating the highly acidogenic nature of the plaque. The inset (upper right) shows plaque sampled from the same area 2 weeks after following a home care program which included lifestyle changes.***

Polyols (hexitols) such as sorbitol, lactilol, mannitol, and xylitol are now used in a variety of products such as chewing gums and confectionery. Sorbitol is a "low cariogenic" sucrose substitute which is used commonly in many "sugar-free" health care products, such as toothpastes, lozenges, syrups and medicines. *Streptococcus mutans* and *Lactobacillus casei* can ferment mannitol and sorbitol, but are inactive towards xylitol[72]. While frequent consumption of sorbitol may result in a degree of metabolic adaptation by dental plaque bacteria, this is probably of no clinical importance, at least for persons with a normal salivary function and for people with a moderate consumption of sorbitol-containing products[73].

Of all the current candidates for sucrose replacement, xylitol has attracted the greatest interest, primarily because of its potential to be an active rather than a passive anti-caries agent[74]. The vast majority of plaque bacteria cannot ferment xylitol into cariogenic acid end-products, nor can they adapt to metabolise xylitol, despite frequent exposure to it[75]. When it is

transported into and accumulates within mutans streptococci, it inhibiting fermentation either by depleting the cell of high-energy phosphate or by poisoning the glycolytic system [76]. Through these pathways, xylitol inhibits plaque growth and acid production, and reduces the number of mutans streptococci. By doing this, xylitol prevents a shift of the dental plaque bacterial community towards a more cariogenic microflora [77,78].

Finally, patients can be advised to reduce their intake of carbonated drinks between meals, and to reduce their habits of adding sugar to tea, coffee or other drinks. They should drink clean water, low fat milk or unsweetened juices. Caries-protective snack foods such as low-fat cheese are a worthwhile addition to the diet.

## Conclusions

Acid production by dental plaque is a key factor in the caries process. Using simple tests this process can be demonstrated to patients and used to screen patients for pathogenic plaque (Figures 3 and 4). Lifestyle changes which reduce plaque acidogenesis for destructive acids, but permit the production of weaker acids with buffering capabilities, can serve to maintain a healthy balance between the plaque biofilm and the tooth surface.

## Disclosure

The author played a major role in developing the Plaque-Check+pH diagnostic kit which was subsequently marketed by the GC Corporation, Japan, and has a commercial interest in this product.

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