

**Title: Evidence for biofilm acid neutralization by baking soda**

**Author:**

Domenick T. Zero, DDS, MS, Indiana University School of Dentistry, Oral Health Research Institute, 415 Lansing St, Indianapolis, IN 46202, [dzero@iu.edu](mailto:dzero@iu.edu)

**Corresponding Author:**

Domenick T. Zero, DDS, MS  
Indiana University School of Dentistry  
Oral Health Research Institute  
415 Lansing St, Indianapolis, IN 46202  
Tel: (317) 274-8822  
Fax: (317) 274-5425  
Email: [dzero@iu.edu](mailto:dzero@iu.edu)

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## **Role of dental biofilm acid production in the caries process**

Dental caries is a complex disease in which there are interactions among the tooth structure, oral microbial biofilm formed on the tooth surface, dietary sugars and to a lesser extent starches, and salivary and genetic influences.<sup>1</sup> Biofilm bacteria metabolize sugars and produce acids, which over time demineralize tooth enamel and can lead to progressive destruction of the tooth's hard tissues and if left untreated pain, abscess and possible tooth loss. The central role of the interaction between dietary sugars and dental biofilm is very well established.<sup>2</sup> However, views on the role of specific organisms, such as *Streptococcus mutans*, in caries causation have changed over time. Several other biofilm microorganism species from the genera Veillonella, Lactobacillus, Bifidobacterium, and Propionibacterium, low-pH non-S. mutans streptococci, Actinomyces spp., and Atopobium spp. with acid producing and acid tolerating properties have been also associated with caries.<sup>3</sup> The emphasis is now on the biofilm as a community of endogenous microorganisms and how ecological conditions, mainly determined by frequent consumption of dietary sugars and low saliva flow (hyposalivation), can shift the biofilm from a healthy state to a caries conducive condition.<sup>4,5,6</sup> This ecological pressure from biofilm acidification leads to adaptation of the endogenous microorganisms to an acid environment which favors more acid tolerant (aciduric) bacteria and increased acid producing potential.<sup>4</sup>

Dental caries is a dynamic process involving cycles of mineral loss (demineralization) and mineral gain (remineralization).<sup>1,7</sup> The tooth surface is in a healthy state of dynamic equilibrium with the local oral environment (mainly created by saliva and the fluid phase of the biofilm) when demineralization and remineralization are in balance. The caries process occurs under oral conditions that favor more net demineralization than remineralization resulting in sustained net mineral loss. The demineralization phase starts with the formation of organic acids, mainly lactic acid, as an end product from sugar metabolism.<sup>6</sup> As acid builds up in the biofilm the pH drops to the point where conditions become undersaturated with respect to tooth mineral (critical pH; approx. 5.5); the lower the pH, the greater the

degree of undersaturation and the greater the rate of demineralization.<sup>8</sup> Under conditions when sugar metabolism is not taking place or acids have been neutralized, the biofilm pH tends to be in the neutral or basic range and the fluid phase of the biofilm is sufficiently saturated with calcium and phosphate ions so that redeposition of mineral (remineralization) is favored. The presence of low levels of fluoride reduces the net mineral loss during acid challenge and greatly enhances the reprecipitation process, which is considered the main mechanism of action for fluoride.<sup>9</sup>

The pH of the biofilm is influenced by many factors including: concentration of the dietary sugars; the composition, thickness and diffusion properties of the biofilm; the bicarbonate concentration in the saliva; and the velocity of the film of saliva as it passes over the surface of the biofilm.<sup>10,11</sup> The decrease in pH and subsequent return to neutrality that occurs when the biofilm is exposed to dietary sugars is commonly referred to as the Stephan curve.<sup>10</sup> Saliva plays an important role in modifying biofilm pH.<sup>11</sup> Salivary flow rate is the main factor affecting the clearance pattern of cariogenic foods and beverages. Saliva also plays an important role in modifying biofilm pH. In the absence of normal salivary flow, the pH stays at a low level for an extended period of time after exposure to dietary sugars (see Figure). Therefore, saliva is responsible for the recovery of biofilm pH back towards neutrality. Stimulated saliva, because of its higher flow rate (increased volume) and enhanced buffering capacity (bicarbonate buffering system), dilutes and neutralizes biofilm acids.

The greater the acid concentration in the dental biofilm the greater the driving force for acid to diffuse into the tooth. The two important factors that influence the amount of enamel demineralization are: the extent of the pH drop below the critical pH and the extent of time the pH remains below the critical pH. Measures that decrease acid production or elevate the pH through acid neutralization will prevent demineralization and favor remineralization and caries prevention.

#### **Direct effects of baking soda (sodium bicarbonate) on biofilm pH**

The potential of caries intervention strategies directed at the main driver of the caries process – acidification of the dental biofilm below the critical pH – have long been a subject of interest. The rapid alkalization of the biofilm by baking soda is one such approach. A previously published review has supported the use of baking soda in dentifrice formulations because of its safety, low abrasivity, and compatibility with fluoride.<sup>12</sup> The high solubility of baking soda makes it ideally suited to rapidly penetrate the dental biofilm and neutralize acids; however, this also limits its ability to have a protracted effect in the oral cavity.<sup>12</sup>

There is limited direct evidence that baking soda in dentifrice can neutralize biofilm acidity when used in association with a dietary sugar challenge. Blake-Haskins and colleagues<sup>13</sup> reported on a series of experiments using three models to study the effectiveness of dentifrices containing bicarbonate as buffering agents to neutralize biofilm acids. The first model involved touch electrode measurements at mesiobuccal sites of maxillary premolars and molars of subjects after 3 days of biofilm growth and two hours of fasting. For the first control test, baseline pH measurements were followed by a 10% sucrose rinse for three minutes and then the pH was monitored for 60 minutes. For the second test, the subjects rinsed with 10% sucrose for three minutes and after a two minute interval rinsed with a 45% weight/weight fluoride/baking soda dentifrice slurry for three minutes and then the pH was monitored for another 60 minutes. The fluoride/baking soda dentifrice slurry treatment resulted in a higher mean  $\pm$  SD minimum pH after sucrose challenge than control,  $5.60 \pm 0.55$  vs.  $4.34 \pm 0.45$  pH units (n=5). This study does provide some evidence of the ability of dentifrice with baking soda to help neutralize biofilm acids; however, individuals do not typically brush their teeth two minutes after a sugar challenge. The second model involved pH telemetry using a partial denture and showed a dose-dependent transient increase in biofilm pH to sodium bicarbonate concentration. The third model involved the extraoral testing of biofilm samples that prior to collection received no treatment, rinsing with fluoride/baking soda dentifrice slurry or rinsing with fluoride dentifrice slurry. After baseline pH measurement the

biofilm samples were challenged in vitro with a glucose solution and the pH recorded for 10 minutes. This somewhat contrived experiment showed that the fluoride dentifrice with baking soda inhibited the pH drop for a glucose challenge more than the fluoride control dentifrice.

Dawes<sup>10</sup> used a well-controlled in vitro test plaque model to study the effects of different dilutions of a fluoride with baking soda (65%) dentifrice and another marketed fluoride dentifrice on the recovery phase of the Stephan curve. The treatments were applied as a dentifrice/saliva slurry for one minute, 20 minutes after a one minute challenge with 10% sucrose when the pH had dropped to about 4.5. The study found that at the higher baking soda concentrations tested (0.5 and 1 mol/L), there was a rapid return towards neutrality which remained elevated for a further two hours. For the control fluoride dentifrice, the pH rose slightly, but after 2 hours it had not reached pH 5.5.

Further evidence of the ability of sodium bicarbonate to rapidly neutralize biofilm acid in subjects with marked hyposalivation has been reported by Meyerowitz & Zero<sup>14</sup>. Individuals with salivary dysfunction have a diminished ability to clear dietary sugars and buffer biofilm acids. Five subjects with radiation induced hyposalivation or Sjögren's syndrome [mean (SD) stimulated saliva 0.06 (0.08) ml/min] were recruited for the study. Biofilm pH was measured using the touch electrode method at baseline and then at 2, 5, 10, 20, 30, 60 and 90 minutes following a one minute 10% sucrose rinse. Following an additional 10% sucrose rinse, the subjects rinsed with a 0.1 mol/L NaHCO<sub>3</sub> solution for one minute and plaque pH was measured again at intervals up to 90 minutes. A similar protocol was followed for five control subjects whose mean (SD) stimulated saliva flow rate was 1.42 (0.55) ml/min. Following the 0.1 mol/L NaHCO<sub>3</sub> rinse the mean (SD) pH in the hyposalivation subjects rose from 5.0 (0.5) to 9.2 (0.1) at five minutes and remained alkaline for 90 minutes. In the control subjects the mean (SD) pH rose from 5.4 (0.3) to 8.5 (0.4) at 2 minutes and rapidly returned towards neutrality. Following a third sucrose challenge the biofilm pH was rapidly depressed again. These results indicate that subjects with marked hyposalivation have protracted depressions of plaque pH following an acidogenic challenge compared

with that observed in subjects with normal salivary flow. However, this can be readily reversed and maintained in the alkaline range for extended periods with a bicarbonate rinse. While not tested in this study, it is likely that baking soda toothpaste can be used in individuals with hyposalivation as a measure to rapidly reverse the biofilm pH drop after sugar consumption; however, it appears that it may be less effective in preventing an acidogenic response when used as a pre-treatment before a dietary sugar challenge.

Other studies have evaluated a fluoride dentifrice with baking soda for its effect on in vitro and in situ demineralization and remineralization, which can be more directly related to the caries process.

Kashket and colleagues<sup>15</sup> using a short-term intraoral demineralization model employing *Streptococcus mutans*-coated blocks of bovine enamel showed that a fluoride dentifrice containing baking soda and peroxide reduced surface enamel demineralization from a 10% sucrose rinse comparable to a control fluoride dentifrice. In a subsequent study with this model, Kasket and colleagues<sup>16</sup> reported that a high concentration baking soda dentifrice reduced sucrose-induced demineralization and that the buffering effect of baking soda was predominant over the effect of fluoride in this model. It should be noted that the sucrose challenge was delayed for 30 minutes after the 20% dentifrice slurry treatment in the former study and 30 or 60 minutes in the latter study to minimize the effect of fluoride in the model. These findings could be applicable for individuals that brushed before a cariogenic meal or snack, although the nature of the model and study design limit clinical inference.

Cury and colleagues<sup>17</sup> evaluated the effect of fluoride dentifrice with baking soda in a longer-term (28 day) in situ biofilm demineralization/remineralization model, which involved two sound enamel specimens (demin model) and two enamel specimens with artificial caries lesions (remin model) held in acrylic palatal appliances of ten subjects. The enamel specimens were recessed from the surface of the appliance by 1 mm and covered with a plastic mesh to encourage biofilm formation. During meal times the appliances were removed and the specimens exposed to a 10% sucrose solution three times per

day. Ten minutes after the sugar challenge, subjects were instructed to apply a slurry of either a placebo, fluoride or fluoride and baking soda (20%) dentifrice to the specimens before reinserting their appliances. Both fluoride dentifrice treatments were found to enhance remineralization compared to placebo as determined by changes in cross-sectional microhardness; however, for the fluoride with baking soda dentifrice, the remin response was only slightly higher than the fluoride positive control dentifrice and did not reach statistical significance. Only the fluoride with baking soda dentifrice showed significantly less demin than the placebo, and although the difference between the two fluoride dentifrices was not statistically significant, it was directionally in favor of the dentifrice with baking soda. It is not clear if this study was adequately powered to show differences between the treatments as no power calculations were included.

In summary, there is evidence that sodium bicarbonate can rapidly elevate an acidic biofilm pH to alkaline levels. There is the potential that daily use after exposure to dietary sugars may be of some benefit in preventing dental caries; however, the timing is critical and it appears that the effectiveness of baking soda in dentifrice is concentration dependent.

### **Effect of baking soda on enamel remineralization**

It is well established that the carbonate content of human enamel apatite is associated with lower crystallinity and higher acid solubility.<sup>18</sup> Several studies have evaluated whether sodium bicarbonate interferes with the remineralization of enamel subsurface caries lesions and their subsequent acid resistance.<sup>19,20</sup> Tanakaa & Iijima<sup>19</sup> showed that the combination of fluoride and bicarbonate increased acid resistance more than each alone. Kuramochi and colleagues<sup>20</sup> found that in the presence of bicarbonate, carbonate ions were incorporated into enamel lesions during remineralization; however, the extent of remineralization or acid resistance was not affected.

### **Effects of baking soda on biofilm composition, metabolic activity**

It is possible that dentifrices with baking soda can influence the cariogenic properties of the dental biofilm by changing its composition and metabolic activity. Based on the ecological plaque hypothesis, interventions that shift the oral ecology towards a more neutral pH should favor tooth health.<sup>5</sup>

Dentifrice with baking soda could help shift the biofilm towards a health-conducive state by interfering with the acid adaptive response of biofilm microorganisms to the low pH environment created by ingestion of dietary sugars and the subsequent selection of cariogenic microorganisms that can tolerate and adapt to the low pH conditions creating a dysbiosis. This may be particularly beneficial to individuals with hyposalivation due to the lack of natural buffering capacity from saliva.

There is some evidence that the use of baking soda dentifrices can alter the composition of the biofilm. Legier-Vargas and colleagues<sup>21</sup> investigated the clinical effects of baking soda (65%) dentifrices with and without fluoride against a placebo dentifrice without baking soda or fluoride. Ten subjects were randomly assigned to one of the three dentifrice treatments using a crossover design over a 4-week period. There were statistically significant reductions in the numbers of mutans streptococci in saliva after using the baking soda dentifrices as compared to the placebo treatment. Although not statistically significant, the numbers of lactobacilli were also similarly reduced. It is quite surprising that this promising finding has not been followed up with larger scale clinical studies given the now wide acceptance of the ecological plaque hypothesis.<sup>6,22</sup> Furthermore, studies on the effect of baking soda on the acidogenic and aciduric properties of the biofilm microorganisms are warranted, especially in individuals with hyposalivation.

#### **Other strategies to control biofilm pH**

In addition to baking soda dentifrices, other approaches to maintain biofilm pH in a healthy range, above the critical pH, should also be considered. Chewing gum containing baking soda is another promising strategy where the addition of bicarbonate to the increased bicarbonate levels in saliva due to gustatory



and mechanical stimulation may be beneficial. Anderson & Orchardson<sup>23</sup> showed that in healthy subjects with normal salivary flow sugar-free gum with baking soda (4%) resulted in a significantly greater salivary pH than the sugar-free control gum over a 30 min test period, while the salivary flow rates with the two gums were not different. It has also been shown that baking soda (2%) in sugar-free chewing gum significantly increased the extent and rate of rise in interproximal plaque pH compared to a control gum when chewed 20 minutes after a sugar challenge (toffee).<sup>24</sup> In addition the elevated pH benefit persisted for the gum with baking soda for at least 20 min after the 10 minute chewing period ended. Chewing the gum with baking soda before the toffee exposure showed only a slight effect on minimum pH compared to the control gum (4.6 vs. 4.4).

Another strategy involves the use of prebiotics like arginine to improve the pH homeostasis by inducing alkali production by the bacterial arginine deiminase system, thus altering the biofilm ecology towards a healthier state.<sup>25,26</sup> There is some evidence that arginine when added to fluoride dentifrice may contribute to caries prevention.<sup>27</sup>

### **Clinical Implications of acid neutralization in controlling dental caries**

While there are many approaches to treat the consequences of dental caries disease process, emphasis is shifting towards measures that preserve teeth.<sup>28</sup> There is the recent recognition that maintaining the dental biofilm in a healthy state by preventing sugar- and hyposalivation-induced biofilm dysbiosis is an underutilized strategy.<sup>22</sup> The acid neutralization of dental biofilm using baking soda-containing fluoride dentifrice has potential for helping to counteract modern high sugar diets by rapidly neutralizing biofilm generated acid. Based on limited available evidence it appears that the benefits of baking soda are concentration-dependent and given the wide range of baking soda concentrations (10-65%) in marketed products this will likely affect their ability to neutralize biofilm acids. The timing of using a baking soda dentifrice in relationship to a dietary sugar exposure is also important. The sooner the better so as to

prevent a sustained biofilm pH drop and demineralization. It is highly likely that individuals with decreased salivary flow may benefit the most; however, this would again depend on the timing of product use in relationship to the sugar exposure. It appears from clinical studies that baking soda, due to its high solubility, has limited substantivity and does not have a marked effect on limiting the biofilm pH drop when used before a dietary sugar challenge. As most individuals only brush once or twice per day and do not brush their teeth after every sugary meal and snack, there is the need for other measures to maintain pH homeostasis throughout the day. Besides limiting exposures to dietary sugars, these could include the use of sugar-free chewing gum and mints with baking soda as well as arginine-containing products.

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