

Clinical Evaluation of the Effect of Nanohydroxyapatite Lozenge on the pH of Dental Plaque

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Purpose: This study investigated the influence of nanohydroxyapatite-containing (nanoHAP) lozenge on plaque pH following sucrose intake.

Patients and Methods: Sixteen adult subjects were enrolled in this double-blind crossover study composed of four interventions: (1) 10% w/v sucrose solution, (2) 10% w/v sorbitol solution, (3) nanoHAP lozenge, and (4) 10% w/v sucrose solution challenge followed by nanoHAP lozenge. Following the determination of each subject's resting plaque pH, the pH was measured at different time intervals from 3 to 30 minutes from the start of intervention, with 7 days interval between the applications of different interventions. The data were analyzed using the analysis of variance and Tukey's test ($\alpha < 0.05$).

Results: While sorbitol produces no change in plaque pH, nanoHAP-lozenge increased the plaque pH from a baseline of 7.0 ± 0.3 (mean $\pm$ sd) to 7.8 ± 0.2 (mean $\pm$ sd) within 30 minutes. Sucrose lowered the plaque pH from a baseline of 7.0 ± 0.4 (mean $\pm$ sd) to the lowest minimum of 5.1 ± 0.1 (mean $\pm$ sd) at the 7th minute, rising above the critical pH of enamel dissolution (5.5) at 12th minute and the baseline pH in more than 30 minutes. With lozenge intervention following sucrose challenge, plaque pH rose to 5.5 in 8 min, and to the baseline pH in 24 min. The cH area (Hydrogen ion concentration area) produced by sucrose (1.82 sq. units) was significantly ($p < 0.05$) greater than that produced when sucrose was challenged with lozenge (0.48 sq. units).

Conclusion: Nanohydroxyapatite-containing lozenge increased plaque pH, reduced plaque pH drop in the presence of sucrose, and facilitated the rapid recovery of plaque pH after sucrose intake.

Keywords: dental plaque, nanohydroxyapatite, demineralization, buffering, lozenge

Introduction

The prevalence of dental caries remains the highest among oral diseases in all age groups and the highest chronic disease among children.¹ Untreated dental caries is observed in 28% and 18% of 35–44 and 65+ years old people, respectively.^{2–5} Dental caries is a biofilm-mediated and diet-modulated oral disease, the process of which is dynamic, with alternating periods of homeostasis and dysbiosis.⁶ Cariogenic bacteria in dental plaque generate their energy for growth through the metabolism of sugars, with consequent production of organic acids.^{7,8} These acids demineralize the underlying tooth tissue by lowering the local plaque pH, resulting in dental caries.^{9,10}

Among the methods that have been used to mitigate the caries process is the application of agents that can buffer acids immediately following the consumption of fermentable sugars. Using chewing gum to increase the alkaline bicarbonate content of saliva by mechanically stimulating and increasing the saliva flow rate has long been used as a physiological strategy for caries control by limiting the frequency of low plaque pH. Some oral hygiene products, such as toothpastes and mouth rinses, have also been used as vehicles to carry either alkali-generating or acid-buffering agents into the oral cavity to neutralize the organic acids in saliva and plaque. Urea or arginine incorporated into any of these products is metabolized by urease and arginine deaminase, respectively, produced by several oral bacteria to generate

ammonia that raises the pH of plaque and helps to neutralize the acid produced by bacteria metabolism.¹¹ Acid production in plaques in response to glucose challenge has been reported to be reduced by the incorporation of zinc ions in snack foods or mouthrinses^{12–14} and by adding triclosan to toothpaste or mouthrinses.^{15,16} Thus, the incorporation of alkali-generating or acid-buffering agents into snack foods, confectioneries, or oral care products has been a physiological strategy to control dental caries in the population, especially among those at high caries risk.

Phosphate (PO_4^{3-}) ions in saliva and oral hygiene products have the potential to enhance the buffering capacity of saliva to neutralize organic acids from bacterial metabolism.^{17,18} Hydroxyapatite (HAP) incorporated into plaque and saliva through the use of oral care products (toothpaste, mouthwash, gels, and dental creams) elevated calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ion concentrations in saliva and plaque, thus serving as reservoir for these ions.^{19,20} It has been demonstrated that under acidic conditions in the plaque, calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions are released as HAP dissociation products,^{21,22} with the released phosphate (PO_4^{3-}) ions contributing to acid neutralization in the plaque in a similar way as the salivary phosphate buffer.^{9,20} One may expect that the released calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions would theoretically bind to promote calculus formation, but the chance of this occurrence is reduced by some proteins, such as statherin, in saliva and plaque.²³ Increased calcium (Ca^{2+}) content in the plaque helps inhibit the dissolution of tooth tissue under acidic conditions.²⁴

It is pertinent to mention that among the above discussed vehicles that have been used to apply alkali-generating or acid-buffering agents into the oral environment, chewing gum is the most favored because of the convenience of being used by individual at any time and at any place. Lozenge is another convenient vehicle that can be used for this purpose. However, it has not been used to mitigate the lowering of plaque acidity following intake of fermentable sugars. For this reason, the present study investigated using lozenge as a vehicle to carry HAP into the oral environment, and hence into plaque and saliva, and to determine its ability to reduce the lowering of plaque pH when applied immediately after carbohydrate intake. It is envisioned that the new lozenge formulation (Apamoist lozenges; Sangi Co., Ltd., Tokyo, Japan) can be used to enhance the saliva buffering capacity in individuals suffering from xerostomia, as well as in those at high caries risk.

Materials and Methods

Subject Population and Recruitment

The Institutional Review Board (IRB) of the University of Texas Health at San Antonio approved the study (approval #20230227HU). This study was conducted in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its later amendments, and in compliance with the International Conference on Harmonization (ICH) Good Clinical Practice Guidelines. Following the satisfaction of the inclusion criteria and obtaining informed consent, 16 adults (8 males and 8 females) in the age range of 18–40 years, from different ethnic and racial backgrounds, with varied socioeconomic and caries risk status were selected for this crossover study. Only subjects at moderate and high caries risk status, determined using the American Dental Association-approved caries risk assessment tool for adults, were enrolled to participate in this study.²⁵

Subjects were enrolled in the study if they were able to read and sign a written informed consent form that explained the study, agreed to give a full medical and drug history (considering that some diseases and drugs affect the saliva flow rate and, hence, affect the pH of the saliva), agreed to visit for a day each week for a study procedure for a total of 5 weeks, had telephone contact for scheduling appointments and monitored adverse effects, and had a minimum of 20 teeth exposed to the oral environment. Enrolled subjects must be able to produce a reduction in plaque pH to <5.5 (or at least a full unit drop in pH from baseline measured at either 3 or 5 minutes) following a one-minute rinse with 10 mL of 10% sucrose solution (w/v) and have normal unstimulated and stimulated salivary flow rate of ≥ 0.2 mL/min and ≥ 0.7 mL/min, respectively, measured under standardized conditions, using methods described by Sreebny and Valdin.²⁶

Excluded subjects were those that had existing or recurrent disease/dental pathology that could affect the assessments, orthodontic appliances, multiple restorations that would interfere with pH evaluation, or excessive gingival inflammation. Subjects who were allergic to lozenge ingredients such as aspartame, individuals with phenylketonuria (PKU), and those using antibiotics in the previous 28 days or any medication that may affect the metabolism of dental plaque or decrease salivation were also excluded. Those who participated in another clinical trial, received an investigational drug within 15

days of the start of the study, were known to be HIV seropositive or experienced intermittent swelling of salivary glands, local disease (oral candidiasis, lichen planus, etc.), or Sjögren's syndrome, were excluded too.

Study Protocol

The participants started the study with a 7-day washout period, during which they were provided with and asked to use the same brand of 1100 ppm fluoride toothpaste (Colgate Cavity Protection Toothpaste, Colgate-Palmolive Co., New York, NY) for their toothbrushing performed twice daily. Following the washout period, subjects reported that morning at our clinical research facility after refraining from routine oral hygiene procedures for 48 hours and avoided eating or drinking for 4 hours, except for drinking water, prior to the visit. Plaque acidity tests were conducted in two distinct phases as recommended at the Conference on "Modern Methods for Assessing the cariogenic and erosive potential of foods".²⁷ In phase one, the plaque pH profiles of the subjects were determined for each of the control solutions and lozenge: (negative control) 10% w/v sucrose solution (Sigma-Aldrich, Inc., St. Louis, MO, USA), (positive control) 10% w/v sorbitol solution (Sigma-Aldrich, Inc., St. Louis, MO, USA), and (experimental) nanohydroxyapatite-containing lozenge (Apamoist lozenges, Sangi Co., Ltd., Tokyo, Japan). The lozenge contains nanohydroxyapatite (2.5%), lactoferrin concentrate, D-sorbitol, Xylitol, Sodium hyaluronate, Monosodium L-glutamate, Erythritol and hydroxypropyl cellulose complex, Aspartame, Hydroxypropyl cellulose, Calcium stearate, Microcrystalline cellulose, Silicon dioxide, L-tartaric and sodium bicarbonate complex, DL-malic acid, Riboflavin, and Powdered fragrance. In the second phase, plaque pH profiles were repeated for the lozenge after the subjects were pre-challenged with 10% sucrose rinse. The test products were administered to the subjects in a randomized crossover design, with a 7-day interval between the use of the products.

Plaque Harvesting and pH Measurement

Before the start of each test cycle to measure the pH of plaque suspension using Sentron 2001 pH meter, the microelectrode pH electrode together with a liquid junction capillary reference electrode (Lazar Research Laboratories, Inc., Los Angeles, CA, USA) was calibrated using pH 4.0 and 7.0 buffers. During the test, pH 7.0 buffer was used to monitor the calibration drift. With the aid of a blunt-pointed nickel microspatula, a pooled sample of plaque was collected over a period of 60 seconds. The plaque was collected from the smooth surfaces of six teeth that represent all quadrants of the mouth, except the lower anterior teeth (to avoid saliva contamination). Plaque that was deposited on restorations, food remnants, and salivary/blood contamination were avoided. Prior to plaque collection, subjects were asked to swallow their saliva as a precaution to avoid saliva contamination. Approximately 1 mg (wet weight) of plaque was collected and made into suspension in 20 μ L of sterile deionized distilled water by 10-second gentle mixing in a prefabricated single-use disposable well, and the pH was recorded after 10s. This regime was adhered to allow the electrode reading to stabilize and obtain a standardized reading of the pH as the metabolism of the plaque continues in the well. The electrode tip was rinsed with sterile deionized distilled water before proceeding to the next reading.

In phase one, the test products' (sucrose, sorbitol, and lozenge) effects on plaque pH were determined for each participant using the Stephan curve model.²⁷ For sucrose or sorbitol, the resting pH of the subjects was determined by plaque sampling immediately before a one-minute rinse with sucrose or sorbitol solution. Subject then received 15 mL of 10% (w/v) sucrose rinse and was asked to rinse the mouth with it for 1 min before expectorating. Plaque pH was measured at 3, 7, 11, 15, 19, 23, 27, and 30 min after the start of the sucrose rinse. This procedure was repeated using 10% (w/v) sorbitol. For the lozenge, the resting plaque pH of the subject was first determined, and then the subject was instructed to suck one lozenge for 30 min, during which the pH of the plaque was evaluated at 3, 7, 11, 15, 19, 23, 27, and 30 min from the start of sucking.

In the second phase, plaque pH responses were determined for the lozenges after a prior challenge with 10% (w/v) sucrose rinse. Subjects were instructed to rinse with 15 mL of a 10% sucrose solution for one minute. Then, after 5 min, each subject was asked to suck on the lozenge tablet for 30 min as previously described. Plaque samples were collected prior to the sucrose challenge and at 5 min post-challenge, and then at 3, 7, 11, 15, 19, 23, 27, and 30 min from the start of the lozenges' sucking, that is, 8, 12, 16, 20, 24, 28, 32, and 35 min from the start of the sucrose rinse.

Statistical Analysis

The statistical analysis was performed with SPSS 17.0 (SPSS Inc., Chicago IL) statistical software, with a significance level pre-chosen at $p < 0.05$ for all statistical tests. The assumptions of equality of variances were verified by normal probability plots. Following this, repeated-measures ANOVA was applied to compare the mean ΔpH (pH difference between the baseline plaque pH and plaque pH at each measurement time point) values for multiple time intervals, while Tukey post-hoc comparison test was used for inter-group comparisons of ΔpH at all measurement time intervals.

Results

Figure 1 depicts the pH at different time intervals for every test product. While the intake of sorbitol rinse did not cause any change in plaque pH, the intake of the lozenge led to a rise in the pH of the plaque from a baseline of 7.0 ± 0.3 (mean $\pm$ sd) to 7.8 ± 0.2 (mean $\pm$ sd) within 30 minutes. Sucrose intake lowered the plaque pH from a baseline of 7.0 ± 0.4 (mean $\pm$ sd) to the lowest minimum of 5.1 ± 0.1 (mean $\pm$ sd) at the 7th minute, and then the pH rose to cross the critical pH for enamel dissolution (5.5) at 12th minute, but never reached the baseline pH at the expiration of 30 minutes. A similar pattern was observed when sucrose rinse was followed by lozenge intake; however, the intervention of lozenge accelerated the recovery of plaque pH to cross the critical pH at 8th minute and reach the baseline pH in 24 min and pH of 7.62 by 30th minute.

Furthermore, Figure 1 shows the hydrogen ion concentration area (cH area), ie, the area enclosed by Stephan's curve below the line representing the critical pH (5.5) at the two points where the pH crossed the critical pH line during its decline and recovery to baseline after intake of either sucrose alone or sucrose followed by lozenge. This area represents the amount of demineralization experienced by a subject with intake of a particular food substance (cariogenicity).²⁷ The cH area was calculated for sucrose and combined sucrose + lozenge for each subject.²⁷ The mean cH area produced by sucrose intake (1.82 sq. units) was statistically significantly greater ($p < 0.05$) than that produced when the sucrose intake was challenged with lozenge intake (0.48 sq. units).

Table 1 shows the results of the intergroup comparisons of the effects of the products on plaque pH. The difference between the effects of Sorbitol and Lozenge was not statistically significant (Table 1). Statistically significant differences ($P < 0.001$) were observed between the effects of Sorbitol and Sucrose, Sorbitol and Combined sucrose + lozenge, Sucrose and Lozenge, Lozenge and Combined sucrose + lozenge, and Lozenge and Combined with sucrose + lozenge (Table 1).

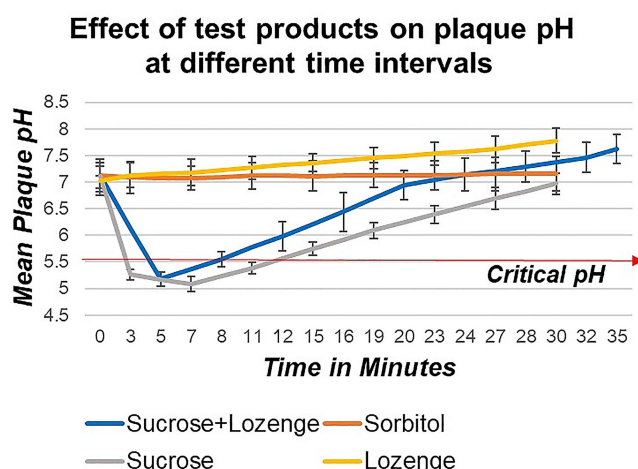


Figure 1 Graphic illustration of the effects of the test products on plaque pH at different time intervals. Lines and bars show means and standard deviations, respectively. The area enclosed by Stephan's curve below the line representing the critical pH (5.5) at the two points where the pH crossed the critical pH line during its decline and recovery to baseline after intake of either sucrose alone or sucrose followed by lozenge is the cH area (Hydrogen ion concentration area). This area represents the amount of demineralization experienced by a subject with intake of a particular food substance (cariogenicity). The mean cH area produced by sucrose intake (1.82 sq. units) was statistically significantly greater ($p < 0.05$) than that produced when the sucrose intake was challenged with lozenge intake (0.48 sq. units). The graph also shows that the sorbitol and the lozenge did not depress the pH below the pH 7 rather both increased the pH above pH 7, with greater increase from the lozenge.

Table I Intergroup Comparisons of the Effects of the Investigated Treatments on Plaque pH

		Mean Difference (I-J)	Std. Error	Sig.*	95% Confidence Interval	
					Lower Bound	Upper Bound
Sorbitol	Sucrose	0.934870*	0.0965011	0.000	0.635561	1.234179
	Lozenge	-0.217000	0.1102167	0.334	-0.544955	0.110955
	Sucrose+Lozenge	0.451150*	0.1121388	0.005	0.118266	0.784034
Sucrose	Lozenge	-1.151870*	0.0791474	0.000	-1.391501	-0.912239
	Sucrose+Lozenge	-0.483720*	0.0818029	0.000	-0.732397	-0.235043
Lozenge	Sucrose+Lozenge	0.668150*	0.0976062	0.000	0.379856	0.956444

Notes: Differences Were Analyzed by the Tukey Post Hoc Multiple Comparisons Test. Based on Observed Means. The Error Term is Mean Square (Error) = 0.047. *The Mean Difference is Significant at the 0.05 Level.

Discussion

Acidogenicity of dental plaque is the driving factor for caries development. Organic acids produced by plaque bacteria demineralize the tooth tissue with manifestation of caries lesions after a long time of mineral loss.²⁸ Although a plethora of oral hygiene (toothpaste and mouthwash) and confectionery (chewing gum) products are currently being used as vehicles to carry either alkali-generating or acid-buffering agents into the oral cavity to neutralize the organic acids in saliva and plaque for dental caries prevention, caries still exists among individuals at high risk because of either low saliva flow rate or poor oral hygiene with high plaque accumulation. Therefore, more effective therapeutic agents are needed. Phosphate (PO_4^{3-}) ions in saliva are known to play a role in neutralizing organic acids from the bacterial metabolism of sugars^{9,20} thus, any material that can increase the plaque and saliva phosphate (PO_4^{3-}) ion concentration may be an effective acid-buffering and caries-preventive agent. It has also been reported that calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions are released in saliva and plaques as dissociation products of nanohydroxyapatite (nanoHAP), particularly under acidic conditions,^{21,22} thus nanoHAP may be an effective acid-buffering agent to neutralize acids produced by bacteria in plaques. In addition to effectiveness, the active agent should be applied via a vehicle that is easily accessible at any time after sugar intake, as well as acceptable by all ages. Although chewing gum has been used to convey buffering agents into the oral environment and is easily carried around by individuals, it is not acceptable for all ages and sexes, particularly adult males.²⁹ Therefore, lozenges were used as vehicles for the active buffering agent in the present study. Based on these facts, this study investigated using a lozenge as a vehicle to carry nanoHAP into plaque and saliva and to determine its ability to combat the lowering of plaque pH when applied immediately after carbohydrate intake. This was performed following the guidelines established at the conference on “Methods for Assessing the cariogenic and erosive potential of foods”.²⁷ Also, plaque harvesting was performed in accordance with Fosdick et al³⁰ and applied by other researchers.^{31–35}

Measurement of plaque pH values and their changes at different time intervals after being challenged with sorbitol or 10% sucrose provided an estimate of the product's acidogenic potential, illustrated as Stephan's curve.³⁶ Stephan demonstrated a decrease in pH levels within dental plaque with sucrose rinse, which was followed by a gradual return to normal levels over time, known as the “Stephan curve”.³⁶ This curve displayed the following three phases: (1) a rapid decline in pH resulting from the metabolism of sucrose by acidogenic bacteria, (2) potential enamel dissolution for the period the pH remains below 5.5, and (3) slow return of pH levels to baseline within 30 to 60 min. Shimizu et al in their confirmation of Stephan's curve reported that the pH decrease after sugar exposure was greater in caries-active individuals than in caries-free individuals.³⁷

In the present study, Stephan's curve was obtained only when the plaque was challenged with a rinse of either sucrose alone or sucrose followed by lozenge (Figure 1). Sorbitol rinse or nanoHAP lozenge did not decrease plaque pH; rather, nanoHAP caused an increase in plaque pH (Figure 1). These findings are not surprising and agree with previous scientific reports on the characteristics of these agents. It has long been established that sorbitol, like other polyol sugars, cannot be metabolized by

acidogenic microorganisms, and as such, sorbitol intake does not result in the production of organic acids by microorganisms to reduce plaque pH.^{38,39} This effect was clearly demonstrated in this study, in which sorbitol rinse failed to cause any change in pH of plaque. The decrease in plaque pH by sucrose intake has long been demonstrated in several studies.^{40–42} Shiva et al reported that a 10% sucrose rinse caused the most prominent drop in pH, with the pH dropping to its lowest level at 10th minute and returning to its baseline level at the 30th minute,⁴² and the observations in this present study are in agreement with their report (Figure 1). However, the increase in plaque pH by nanoHAP can be attributed to the reported release of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions from nanoHAP, particularly under acidic condition.^{19,20} Hydroxyapatite (HAP) incorporated into plaque and saliva through the use of HAP-based products (toothpaste, mouthwash, gels, and dental creams) has been demonstrated to raise the calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ion concentrations in saliva and plaque, thus serving as a reservoir of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions.^{19,20} When acids are produced by bacteria in the plaque, Ca^{2+} and PO_4^{3-} ions are released as HAP dissociation products,^{21,22} and the PO_4^{3-} ions are able to buffer acids to a certain level in similar manner as the salivary phosphate buffer.^{9,20} Moreover, it has long been reported that calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions dissolved from the HAP of the tooth tissue during acidic conditions ($\text{pH} \leq 5.5$) in plaque play a pivotal role in the recovery of the plaque pH to neutrality following the intake of fermentable carbohydrates.⁴³ Furthermore, it has been established that the Ca^{2+} ions react with water molecules to form $\text{Ca}(\text{OH})_2$ that raises the plaque pH, while PO_4^{3-} ions, like the salivary phosphate buffer system, also increase the plaque pH.^{44,45} Also, a previous study by Bayrak et al reported an increase in calcium (Ca^{2+}) and phosphate (PO_4^{3-}) levels in dental plaque with the application of HAP.⁴⁶ Thus, the lessening of the drop in plaque pH when sucrose rinse was followed by nanoHAP lozenge, and the rapid recovery of the pH back to baseline can also be attributed to the elevation of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions in plaque by dissociation of the nanoHAP in lozenge.

It is of interest that previous studies demonstrated the interaction of HAP particles with dental plaque and tooth surface,⁴⁶ thus the plaque may serve as a reservoir for these particles to be dissociated under acidic conditions, resulting in release of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions. For this reason, it is believed that calcium (Ca^{2+}) and phosphate (PO_4^{3-}) levels in plaque and/or at the tooth surface can be increased through the use of oral care products that are based on HAP, and using these products can be an effective preventive strategy to positively influence the demineralization-remineralization processes in vivo.^{19,47–49} In the present study, intervention with nanoHAP-containing lozenges 5 min after 10% w/v sucrose challenge not only arrested the dropping of plaque pH but also initiated the pH recovery towards the baseline immediately into the 3rd minute of Lozenge intervention (Figure 1).

Stephan recommended that classifying the pH values according to a “hypothetical critical decalcifying pH level” is the most significant way to relate pH changes to the caries activity of different groups of individuals. The concept of “critical pH” emanated from the understanding that the dissolution of enamel only begins when pH decrease to 5.5 in plaque.³⁶ However, Dawes⁵⁰ pointed out that some other factors play role in determining the dissolution of enamel, with the levels of calcium, phosphate, and fluoride in plaque fluid being the major determining factors,⁵¹ and these may vary among individuals and between teeth.³⁶ Thus, the use of nanoHAP lozenges will not only buffer the acid in the plaque but will provide the mineral ions needed to inhibit demineralization and promote remineralization.

Following the demonstration of the Stephan’s curve, several investigators have picked interest in the “area enclosed by the Stephan’s curve under the critical pH line” (Figure 1), and not just the minimum pH values recorded. This refers to the part of the curve where the pH remains below the “critical” pH, which is referred to as the hydrogen ion concentration (cH) area (or the area of demineralization) and represents the amount of demineralization that occurred in the tooth tissue underneath the plaque at that moment. In the present study, the cH area for Sucrose alone was 1.82 square unit as against 0.48 square unit recorded when the sucrose rinse was challenged with nanoHAP lozenges (Figure 1). This indicated that nanoHAP limited the drop in plaque pH and facilitated rapid recovery of the pH above the critical pH and to the baseline level. This emphasizes that the longer the pH stays below the critical pH, the greater the demineralization of the tooth tissue; hence, lozenge intervention reduced the amount and period of demineralization, and as such, can possibly serve as a tool to prevent caries development.

Undoubtedly, the inorganic and organic components of saliva that naturally restore salivary pH following carbohydrate intake play a role in pH recovery, especially when all the products tested are expected to stimulate increased salivary flow rate. Saliva possesses buffering properties that are unrelated to the oral hygiene of the individual but may

likely be dependent on the genetics of healthy individuals. The amount and the rate of flow of saliva in the mouth affect both the saliva buffering capacity and acid diffusion from plaque.²⁶ It is well established that plaque in the mouth is subjected to the alternating flow rate of saliva, which contains substrates and buffers (eg, bicarbonate) that clear metabolites.^{52,53} Besides mucins and other glycoproteins, which are the major substrates in saliva, the substrates that contribute to the buffering actions of saliva include proteins, peptides and urea.^{54–58} It is known that variations in the composition and flow rates of oral fluid as well as the metabolic activities of oral bacteria immensely affect the resting pH of plaque.⁴²

It is pertinent to mention that lozenges containing sucrose or sorbitol would significantly increase the flow rate of saliva, and the effect of an increased flow of saliva on a plaque has been reported to include increased availability of nitrogenous substrates to the plaque bacteria and a decreased availability of sugar through fast clearance. With these factors, the plaque would be at a more alkaline level at any given time with variation in its flora content.⁵⁹ Besides, saliva contains a powerful pH-boosting factor, Sialin, that influences the buffering capacity of the plaque fluid, but its contribution to the saliva buffering, which is based on its chemical byproducts (putrescine and ammonia), accounts for only 0.6% of the total saliva buffering capacity.⁶⁰ Bicarbonate accounts for nearly 85% of the total buffer capacity of saliva, with phosphate (PO_4^{3-}) and proteins contributing about 15%.⁶¹

Although carefully performed, the present study was not short of limitations, one of which was the limited number of subjects. A large-scale randomized clinical trial involving geriatric patients and patients with xerostomia as well as individuals at a high caries risk is needed to investigate the influence of nanoHAP lozenge on caries prevention, remineralization of initial caries lesions, and improvement of saliva flow rate in patients suffering from dry mouth.

Conclusion

Using sugar-free nanoHAP-containing lozenge increased plaque pH, minimized the drop in plaque pH with sucrose intake, and facilitated rapid recovery of plaque pH to neutrality, thereby minimizing the size of the cH area, which represents the extent of demineralization. Thus, this study demonstrated the potential of nanoHAP lozenge as a useful device for dental caries prevention.

Acknowledgment

We are grateful to Sangi Company for providing the lozenges used in this study.

Disclosure

All authors report no conflicts of interest in this work.

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