Journal of Nepal Dental Association (2010), Vol. 11, No. 2, Jul.-Dec., 131-136

Original Article

Effects of sodium bicarbonate rinses on dental plaque pH and selective oral micro-organisms in radiated head and neck cancer patients

Kharel A[1], Theerathavaj S[2], Thaweboon S[3], Choonharuangdej S[4]

[1]Instructor, Maxillofacial Prosthetic Service Unit, [2]Assistant Professor, Maxillofacial Prosthetic Service Unit, [3,4]Associate Professor, Faculty of Microbiology, Faculty of Dentistry, Mahidol University, Thailand

Abstract

Introduction: Radiation therapy to head and neck region may lead to decreased salivary flow with subsequent reduction in the buffering capacity and alteration of oral microflora, and eventual increased risk of radiation-induced caries.

Aims & Objectives: The purpose of this longitudinal study was to investigate the alterations in a select group cariogenic microflora, and dental plaque pH in ten patients with radiation therapy and ten healthy controls, following 1M sodium bicarbonate rinses.

Materials and methods: Over a period of 14 days each, the test and the control groups were requested to rinse their mouths with either sodium bicarbonate, distilled water or they went through a rinse free period. After each period they were recalled to the clinic and plaque pH measurements were made at baseline and after 10, 30 and 60 minutes following a sucrose rinse. Microbial cultures were performed to assess levels of mutans streptococci, lactobacilli and the total bacterial count.

Results: The results showed no significant difference in the plaque pH profile after using sodium bicarbonate rinses in both the irradiated and healthy subjects. There was a significant increase in lactobacilli counts between the control and experimental groups for all the phases ($p<0.05$).

Conclusion: The baseline plaque pH in irradiated subjects did not show higher acidity compared to healthy individuals although a delayed period of pH recovery of to neutral levels was noted. Following sodium bicarbonate rinses, there was an increased Lactobacilli count without any sustainable effect on the plaque pH. Further research is warranted to evaluate the shifts in oral microbial ecology in head and neck irradiated patients and the effect of palliative measures such as sodium bicarbonate rinses on oral flora of these patients..

Key words: Dental Plaque, Radiation, Saliva, Oral Microflora

Introduction

Oral cancer is the eighth leading cancer in the world among males and thirteenth among females[1]. The condition is essentially managed by surgery, radiation and chemotherapy, either in isolation or in combination. Radiation in particular has adverse side effects and may manifest as xerostomia, mucositis, dysphasia, superficial burns during the radiation phase and, late consequences as radiation induced caries and osteoradionecrosis[2,3,4].

Caries in irradiated patients is an indirect consequence of decreased salivary secretion, flow rate and consequent reduction in the buffering capacity, all leading to a perturbation in the oral microflora. An increased level of cariogenic microorganisms and a more acidic plaque predisposes to an increased risk of developing radiation induced caries[2,5,6,7].

Sodium bicarbonate mouth rinses haves been advocated for the management of radiation induced caries, to help to sustain the pH balance of the oral cavity. This commonly available and relatively inexpensive chemical compound possesses good buffering capacity to neutralize the acidity of a low pH environment, in addition to its antimicrobial activity against oral pathogens[8- 12]. However, there is

Correspondence

Dr. Aayush Kharel, Maxillofacial Prosthetic Service Unit, Faculty of Dentistry, Mahidol University, 6th Yothi Street, Rajvithi, Bangkok-10400, Thailand, E-mail: aayush864@hotmail.com

scant data on the effect of sodium bicarbonate rinses on the plaque pH and oral micro-organisms in irradiated head and neck cancer patients[13].

The purpose of this study, therefore, was to compare the dental plaque pH profiles and the changes in a target group of cariogenic microflora, in irradiated oral cancer patients and a healthy control age and sex matched, healthy radiation-free subjects, both before and after using sodium bicarbonate oral rinses.

Materials and methods

A total of ten patients out of 180 maxillectomy patients rehabilitated at the Maxillofacial Prosthetic Service, Mahidol University, Thailand were randomly selected for the study. They had a mean age of 43.4 years (Range 19-65 years).

The patient selection criteria included the dose, field and, the date of completion of radiation therapy; all patients selected were one year post radiation therapy. The control, healthy group comprised six males and four female age and sex matched adults (mean age= 32.5 years). The exclusion criteria were patients on antibiotics during the three preceding months, those with tumor recurrence, a previous history of radiation therapy and other systemic diseases such as diabetes.

The study comprised three consecutive phases namely untreated, distilled water treated and sodium bicarbonate treated phases. Oral prophylaxis in the form of scaling was performed for both patients and controls prior to the beginning of the study. Patients were instructed to abstain from tooth-brushing for two days prior to the appointment of each phase.

The study began with a request not to brush for 2 days before the appointment to allow for adequate plaque accumulation. On the appointment day, patients were instructed not to eat or drink for 2 hours before sample collection. Plaque was collected for pH measurement and microbial culture. Then each patient was given 10 ml of 10% sucrose solution and instructed to rinse the mouth for 60 seconds. Following a 10 min, 30 min and 60 min intervals, plaque was collected from a specific tooth for pH measurement by the same examiner. This procedure was repeated for each phase. The experimental protocol is shown diagrammatically in Fig 1.

Over a period of 14 days each, the test and the control groups were requested to rinse their mouths with sterile distilled water or sodium bicarbonate as shown in Fig 1. Thus the total experimental period comprised 30 days. Initially in the untreated phase where patients were asked not to brush for 2 days and the baseline culture and pH measurements were performed 2 days after a brush-free period. This was followed by 2 weeks of sterile distilled water and 2 weeks sodium bicarbonate rinses (Fig 1). After each period they were recalled to the clinic for plaque pH measurements and microbial culture.

A full mouth scaling was done for all patients in the test and control groups after the completion of each phase.

In the first phase of the study all patients were instructed to rinse the mouth with 10 ml of sterile distilled water twice daily for two weeks. During this two week period, the subjects were instructed to brush their teeth with the toothbrush and dentifrice provided. There was no restriction in diet allowing the subjects to follow their regular dietary habits.

Two days prior to the second appointment, subjects were instructed to abstain from tooth-brushing but continue to use the assigned mouth rinse only. Participants in the study were given the same type of dentifrice and toothbrush for maintaining oral hygiene during the two weeks of each phase. Oral hygiene maintenance was reinforced to all participants. After the second appointment, sodium bicarbonate solution of 1M concentration was used for a period of two weeks. All measurements were performed by a single examiner.

The study protocol was approved by the Ethics Committee of Mahidol University (MU 2007-174). A written consent form was obtained from the participants.

Microbiological Analysis

After collection, dental plaque was dissolved in 100 µL of 0.9% NaCl solution and kept in a microtube (Treff Lab, Switzerland). A ten-fold dilution was prepared using 900µl of 0.9% NaCl solution. To ensure even distribution of plaque, the microtube containing dissolved plaque solution was placed on top of vibrator (Vortex Genie 2, Scientific Industries, USA) for 5 seconds before diluting in NaCl solution. Subsequent dilutions were prepared in the range of 10^{-1} to$10^{-7.}$

A dilution of 10^{-2}, 10^{-1} and undiluted solution for both the control and experimental groups were used to culture *Lactobacilli* using Rogasa agar. A dilution of 10^{-2}, $10^{-3}$and 10^{-4} for the experimental group and 10^{-2}, 10^{-1} and undiluted for the control group was used to culture mutans streptococci using MSB agar. A dilution of 10^{-5}, 10^{-6} and 10^{-7} was used for both the control and experimental groups for total bacterial count using BHI agar.

Twenty five µL of varying dilutions were placed in sections of the culture plate. The solution was allowed to dry after which the culture media plates were incubated in a CO_2 incubator (Heraeus, Germany) at 5% CO_2 for 48

hours. The colony morphology of the micro-organisms were examined under a stereomicroscope (Leica G26E, Germany) followed by manually counting the number of colony forming units.

Statistical Analysis

Data from the three different phases namely untreated, distilled water-treated and sodium bicarbonate-treated were used to compare the profile of plaque pH and the level of mutans streptococci, lactobacilli including total bacterial number. Two Way ANOVA or Friedman test was used to test the significant difference ($p<0.05$) for the plaque pH within the control and experimental group. Student's *t* test or Mann- Whitney U test was used to find out the significant difference for the plaque pH between the control and experimental group. SPSS program was used for statistical analysis.

One way ANOVA or Kruskal Wallis test was used to determine the significant differences in the microbial count within the control and experimental group at $p<0.05$. Student's *t* –test or Friedman test was used to find the significant difference of microbial count among the control and experimental group.

Results

Plaque pH

Plaque pH in irradiated patients demonstrated slower recovery to baseline levels for all the three phases in comparison to the healthy control group, after the sucrose rinses. Even though the experimental group showed a slower recovery rate at 60 minute interval for the untreated and distilled water phases, the use of sodium bicarbonate for the experimental group showed a significantly slower recovery rate at both 30 and 60 minutes interval (Fig 2, 3).

Plaque pH of irradiated patients on sodium bicarbonate rinses for two weeks demonstrated significantly slower recovery to base line levels at both 30 and 60 minutes after the sucrose rinses, in comparison to the healthy control group. This trend was also seen when the patients were given distilled water rinses for two weeks or, no rinses.

Table 1: Microbial counts of mutans streptococci, lactobacilli and total bacterial count in the control and the experimental groups for the untreated, distilled water and sodium bicarbonate phases.

	Control Group			Experimental Group		
	Untreated	**Distilled Water**	**Sodium Bicarbonate**	**Untreated**	**Distilled Water**	**Sodium Bicarbonate**
MS	2.207±1.12	2.584±0.77	2.036±1.08	2.63 ± 2.71	2.214±2.16	1.466±1.90
LB	0.115±0.36[a]	0.516±0.70[b]	0.11±0.35[c]	1.119±1.22[a]	1.1± 0.546[b]	2.028±1.01[c]
TBC	6.692±1.27	7.439±1.12	5.06±3.58	5.755±2.06	5.302±2.83	5.731±2.15

[a,b,c] Significantly different at $p<0.05$ (MS= mutans streptococci, LB= lactobacilli, TBC= total bacterial count)

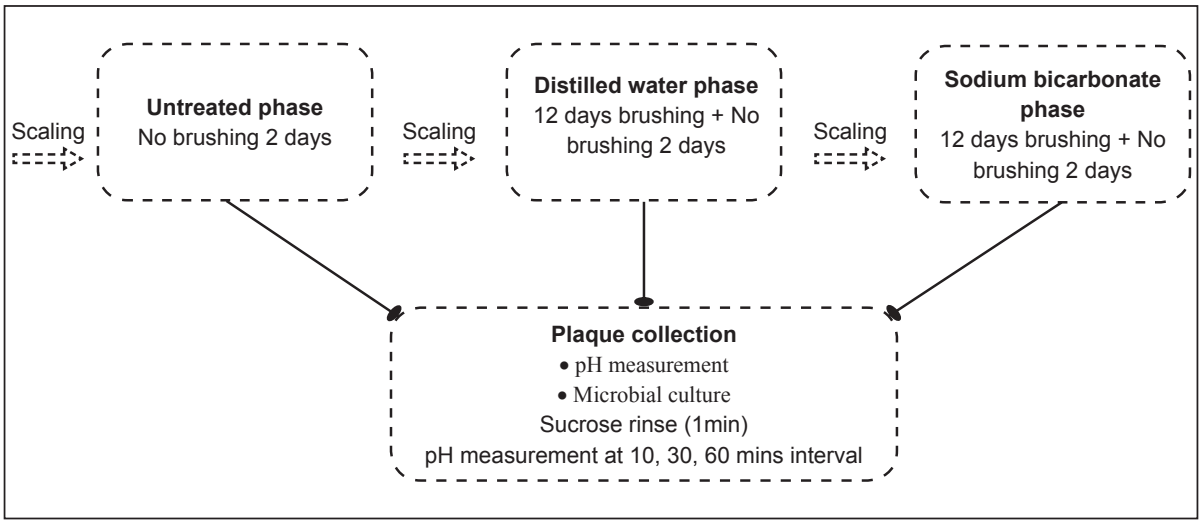

Fig 1: Schematic diagram of the study design

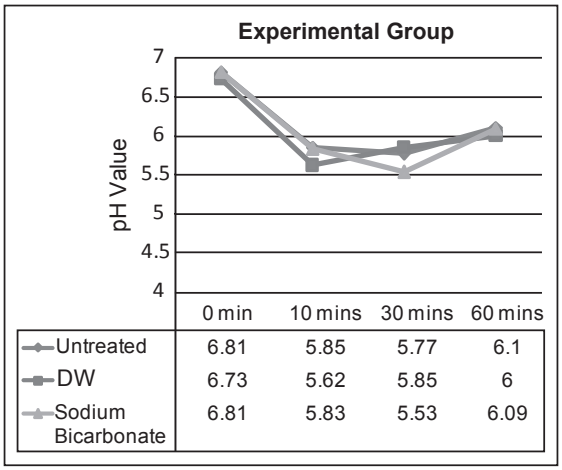

Fig 2: Plaque pH values of the experimental group at baseline, 10, 30 and 60 minutes interval for untreated, distilled water and sodium bicarbonate phases.

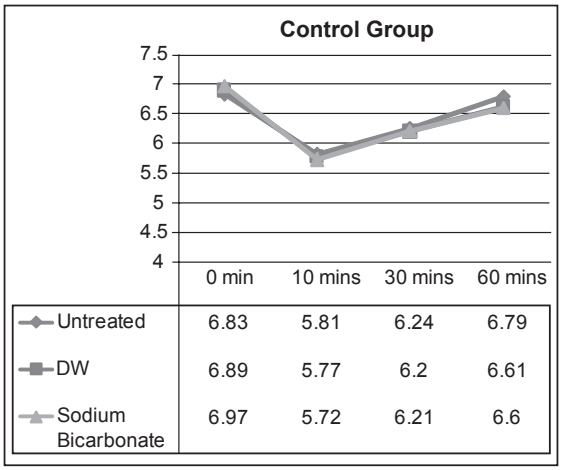

Fig 3: Plaque pH values of the control group at baseline, 10, 30 and 60 minutes interval for untreated, distilled water and sodium bicarbonate phases.

Microbiology

Patients with radiation therapy had a higher count of lactobacilli compared to the control group for all three phases (Table 1). There was a significant difference in the Lactobacilli counts between the control and the test groups for all three phases ($p<0.05$). The number of mutans streptococci decreased after using sodium bicarbonate rinse for two weeks in both the control and the test group.

There were no significant differences in the numbers of mutans streptococci, lactobacilli and total bacterial counts within each group for the untreated, distilled water and sodium bicarbonate treated phases.

Discussion

Under normal physiological conditions, the pH in the saliva ranges from 6.5 to 7.4. Sufficient salivary flow provides the oral cavity with neutralizing components to maintain its buffering capacity[14]. One adverse side effect of radiation therapy to the head and neck region is its effect on salivary gland parenchyma leading to quantitative and qualitative changes in saliva. The salivary output under these circumstances depends upon the total dose and the field of radiation along with volume of salivary gland tissue irradiated[15,16].

Our experiments show reduced salivary flow in irradiated subjects compared with the healthy group, as described in previous studies[17,18,19]. The salivary flow rate impacts upon the buffering capacity which in turn is dependent on the amount of bicarbonate ions in saliva[4,5,6,18]. A decreased salivary flow rate leads to reduced buffering capacity and the overall pH decreases from 7 to 5 in irradiated patients. This in turn prolongs the acidic environment, particularly in dental plaque bioflims, leading to a slow recovery of the pH in irradiated patients as compared to normal patients[3,14,19].

Besides salivary flow and buffering capacity the pH is influenced by diet pattern and oral hygiene that varies among individuals. An increased intake in carbohydrates especially in the form of glucose and sucrose leads to sustained acid production by bacteria and low pH environments in plaque. There was no restriction or modification of dietary habits in subjects participating in this study. In practical terms, the diet pattern tends to vary among individuals and cannot be entirely controlled, hence the latter approach was chosen for the current study.

From this study, we found that the baseline plaque pH of control and experimental subjects was similar for all the three phases. The minimum time elapsed after radiation therapy was one year in our irradiated subjects. Our results agree with the previous work of Moller et al[15] who observed a temporary decline in pH upto three months followed by recovery to neutral levels within one year after completion of radiation therapy. After challenging with a sucrose rinse, it took approximately 60 minutes for the plaque pH of experimental group to revert to the baseline pH compared with shorter time interval for the control group. However, the prolonged acidic plaque pH profile observed may not be solely due the salivary buffering capacity but also on the quantity and the quality of microflora colonizing plaque. This concept was confirmed by the significant increment of S. mutans and Lactobacilli counts in the experimental group compared to the controls.

The presence of higher number of MS and Lactobacilli in experimental group than the control group at untreated phase maybe the consequence of relatively low salivary flow rate combined with reduced buffering capacity of saliva. This condition favors the survival of such aciduric micro-organisms such as MS and Lactobacilli that are capable of producing more acid when exposed to sucrose[5,6]. This finding is consistent with previous data form irradiated subjects showing increased levels of S. mutans, Lactobacilli, Staphylococcus, Actinomyces and S. mitis and a decrease in S. sangius, Neisseria, Fusobacterium, Corynebacterium[5]. In addition, another possibility in increased levels of MS maybe the presence of high concentrations of salivary proteins such as immunoglobulins (IgA, IgG), found in irradiated patients that may in turn promote the binding of S. mutans to the tooth surfaces[14].

Sodium bicarbonate is an inexpensive, non toxic and widely available material. It is used as a major ingredient in dentifrices to remove stain, plaque and maintain the oral hygiene by elevating the pH of the oral cavity by its buffering action[8, 20]. The buffering action is due to the presence of bicarbonate ion. In this study, we used ACS reagent grade sodium bicarbonate in the form of rinse at 1 M concentration that has been topically used in the form of saturated solutions by health professionals[11].

The results clearly showed that neither sodium bicarbonate nor distilled water rinses for two weeks had an effect on the plaque pH profile in comparison to the baseline values. This finding indicates that sodium bicarbonate rinse is unable to neutralize acidic plaque of experimental group and bring the pH profile upto the normal profile found in control group, after a sucrose challenge. This may imply that 1.0 M sodium bicarbonate rinse does not contain sustainable buffering capacity when the regimen of rinsing twice a day for a two week period is instituted.

The antibacterial activity of sodium bicarbonate in addition to its buffering capacity is another area worthy of investigation. A decrease in the amount of MS was noted after using sodium bicarbonate rinses in irradiated subjects, while the reverse was observed in subjects without radiation, both in the control and the experimental groups. The current data correspond to an in-vitro study done by Newbrun et al[9] who found that exposure of S. sangius and S. mutans to 1M of sodium bicarbonate inhibited their growth at 300 mmol/L and showed a bactericidal effect at 600mmol/L. The decreased MS count in control and experimental groups maybe due to the bactericidal effect of sodium bicarbonate used at 1M concentration.

In contrast, higher counts of Lactobacilli were observed in the experimental group after using sodium bicarbonate rinses. A similar phenomenon was observed by Persson et al[13] in elderly hyposalivated patients on medication. The presence of Lactobacilli which are aciduric and acidogenic, in large numbers indicates an acidic environment. Thus the delayed pH recovery and lower pH values of plaque at 30 minutes time interval in experimental subjects treated with sodium bicarbonate rinses implies that the latter procedure disturbed the microbial ecology of irradiated subjects thus leading to an increase in the level of Lactobacilli. Besides mutans streptococci, there are non-mutans streptococci that comprise a major part of dental plaque microflora[21,22] at a pH of 5.0 that may play a significant role in shifting the dental plaque pH towards an acidic environment, as they too are acid tolerant and acidogenic in response to environmental changes[23, 24].

Nevertheless, the use of sodium bicarbonate did not alter the level of Lactobacilli in non- irradiated subjects. This suggests that the composition of microbial community in plaque may be different between irradiated and non-irradiated subjects. It would be interesting to find out the variety of micro-organisms other than MS and Lactobacilli colonizing plaque of irradiated patients.

No sustainable buffering capacity of sodium bicarbonate and distilled water has been observed in this study, although an immediate effect of this solution was noted in both the experimental and control groups. The result indicated that exposure to a sodium bicarbonate rinse, immediately after a sucrose challenge leads to a rapid recovery of pH to neutral level in both groups. However, replacement with distilled water showed no effect on the pH recovery in irradiated subjects. A short term exposure to sodium containing chewing gum can lead to a rapid recovery of pH to neutral levels[25]. Thus, sodium bicarbonate solution seems to have a short post-contact buffering capacity.

Conclusion

Irradiated head and neck cancer patients have a reduced salivary buffering capacity compared to normal healthy individuals.. Sodium bicarbonate rinse is highly effective for short post-contact buffering capacity to elevate the pH to neutral levels. However, it does not induce a sustainable buffering effect on the plaque pH profile when employed as a mouth rinse, twice daily for two weeks regimen. The antibacterial activity helped reduce levels of cariogenic Mutans streptococci with simultaneous increase in levels of Lactobacilli. This indicates that the oral ecology in irradiated patients is significantly different from normal healthy individuals. This highlights the need to have an indepth understanding of the oral ecology in irradiated patients when developing new products intended for preventive oral care. Therefore,

further research should be performed to investigate the spectrum of micro-organism other than MS and Lactobacilli colonizing dental plaque of head and neck irradiated patients.

References

1. Parkin DM, Bray F, Frealey J, Pisani P. Global cancer statistics 2002. Canc J Clin 2005;56:74-108.
2. Beumer J, Curtis TA, Marunick MT. Maxillofacial rehabilitation: Prosthodontic and surgical considerations. Ishiyaku Euro America, Inc. St Louis 1996.
3. Cooper SJ, Fu Karen, Marks J, Silverman S. Late effects of radiation therapy in the head and neck region. Int J Rad Onco Biol Phys 1995;31(5):1141-1164.
4. Vissink A, Jansma J, Spijkervet FKL, Burlage FR, Coppes RP. Oral sequeale of head and neck radiotherapy. Crit Rev Oral Biol Med 2003;14(3):199-212.
5. Brown LR, Dreizen S, Handler S, Johnston DA. Effect of radiation-induced xerostomia on human oral microflora. J Dent Res 1975;54:740-750.
6. Brown LR, Dreizen S, Daly TE. Interrelations of oral microorganisms, immunoglobulins, and dental caries following radiotherapy. J Dent Res 1978; 57:882-893.
7. Almstahl A, Wikstrom M. Oral microflora in subjects with reduced salivary secretion. J Dent Res 1999;78(8):1410-1416.
8. Blake JC, Gaffar A, Volpe AR, Banoczy J, Gintner Z. The effect of bicarbonate/ fluoride dentifrices on human plaque pH. J Clin Dent 1997;8(6):173-177.
9. Newburn E, Hoover C, Ryder M. Bactericidal action of bicarbonate ion on selected periodontal pathogenic microorganisms. J Periodontology 1984; 55(11):658-667.
10. Drake D. Antibacterial activity of baking soda. Compend Contin Educ Dent Suppl 1996;17(19):S17-21
11. Miyasaki KT, Genco RJ, Wilson ME. Antimicrobial properties of hydrogen peroxide and sodium bicarbonate individually and in combination against selected oral, gram negative facultative bacteria. J Dent Res 1986;65(9):1142-1148.
12. Corral LG, Post LS, Montville TJ. Antimicrobial activity of sodium bicarbonate. J of Food Science 1988;53(3):981-982.
13. Persson A, Lingstrom P, Bergdahl M, Claesson R. Buffering effect of a prophylactic gel on dental plaque in institutionalized elderly. Gerodontology 2007;24:98-104.
14. Bardow A, Moe D, Nyvad B, Nauntofte B. The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. Arch Oral Biol 2000;45:1-12.
15. Moller P, Perrier M, Ozsahin M, Monnier P. A prospective study of salivary gland function in patients undergoing radiotherapy for squamous cell carcinoma of the oropharynx. Oral Surg Oral Pathol Oral Radiol Endod 2004;97:173-189.
16. Frank RM, Herdly J, Philippe E. Acquired dental defects and salivary gland lesions after irradiation for carcinoma. J Am Dent Assoc 1965;70:868-883
17. Valdez JH, Atkinson JC, Ship JA, Fox PC. Major salivary gland function in patients with radiation-induced xerostomia: flow rates and sialochemistry. Int J Radiat Oncol Biol Phys 1993;25:41-47.
18. Eliasson A, Carlén A, Almståhl A, M. Wikström, Lingström P. Dental plaque pH and micro-organisms during hyposalivation. J Dent Res 2006;85(4):334-338.
19. Lenander LM, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000; 14:40-47.
20. Newburn E. The use of sodium bicarbonate in oral hygiene products and practice. Compend Contin Educ Dent Suppl 1996;17(19):S2-7.
21. Sansone C, Van H J, Joshipura K, Kent R, Margolis HC. The association of mutans streptococci and non-mutans streptococci capable of acidogenesis at a low pH with dental caries on enamel and root surfaces. J Dent Res 1993;72:508-516.
22. Van H J, Lopman J, Kent R. The final pH of bacteria comprising the predominant flora on sound and carious human root and enamel surfaces. J Dent Res 1996;75: 1008-1014.
23. Svensater G, Borgstrom M, Bowden GHW, Edwardsson S. The acid tolerant microbiota associated with plaque from initial caries and healthy tooth surfaces. Caries Res 2003;37:395-403.
24. Takahashi N, Yamada T. Acid-induced acid tolerance and acidogenicity of non-mutans streptococci. Oral Microbiol Immunol 1999;14:43-48.
25. Igarashi K, Lee KI, Schachtele CF. Effect of chewing gum containing sodium bicarbonate on human interproximal plaque pH. J Dent Res 1988;67(3):531-535.