Published online: 2019-09-30

In Situ Investigation of the Remineralizing Effect of Saliva and Fluoride on Enamel Following Prophylaxis Using Sodium Bicarbonate

Gladis Benjamina Grazziotin[a]
Daniela Rios[b]
Heitor Marques Honório[b]
Salete Moura Bonifácio Silva[b]
José Eduardo Oliveira Lima[b]

ABSTRACT

Objectives: This *in situ* study evaluated the effect of saliva, associated or not with fluoride, on enamel previously submitted to prophylaxis using sodium bicarbonate.

Methods: The study was conducted on enamel blocks submitted to *in vitro* prophylaxis using sodium bicarbonate. The blocks were randomly divided into 2 groups (G1/G2) and mounted on intraoral appliances wore by 10 volunteers. G1 blocks were directly exposed to saliva *in situ*, while blocks in G2 were exposed to saliva with fluoride (rinsing with 0.2% NaF solution during the initial minute). Enamel alterations were evaluated using surface microhardness and profilometry. Enamel hardness data were analyzed by ANOVA and Tukey tests and surface wear was evaluated using paired t test ($P<.05$).

Results: No significant differences were found between G1 and G2 for enamel hardness and wear. The wear after prophylaxis was not different from the wear after the *in situ* stage. Baseline mean values of enamel hardness, after prophylaxis and after the *in situ* stage were 340±16.6, 329±35.7 and 354±37.8 for G1 and 338±15.6, 312±46.3 and 340±21.8 for G2, respectively.

Conclusions: It was concluded that saliva alone exhibited a similar effect to saliva associated with fluoride; after 4h of *in situ* remineralization, there was no recovery in height of the enamel structure that had been lost due to the application of sodium bicarbonate. (Eur J Dent 2011;5:40-46)

Key words: Dental prophylaxis; Saliva; Fluoride; Wear; Dental enamel.

[a] Department of Pediatric Dentistry, Santa Cruz do Sul University, Santa Cruz do Sul -RS, Brazil.
[b] Department of Pediatric Dentistry, Orthodontics and Public Health, Bauru School of Dentistry, USP - University of São Paulo, Bauru-SP, Brazil.

Corresponding author: Heitor Marques Honório
Faculdade de Odontologia de Bauru-USP.
Departamento de Odontopediatria, Ortodontia e Saúde Coletiva. Alameda Octávio Pinheiro Bizolla, 9-75 - Bauru- SP- Brasil, 17012-901.
Phone: + 55 14 3235-8256
E-mail: heitorhonorio@usp.br

INTRODUCTION

Dental caries has been widely investigated for decades.[1,2] Knowledge of the multifactorial etiology of caries concerning cariogenic diet, frequent ingestion of sucrose, and presence of dental biofilm on the dental surface of a susceptible host for a certain period of time encouraged investigations into preventive methods.[2-4]

Initially, classical studies demonstrated that dietary control with reduced ingestion of sucrose leads to a lower prevalence of dental caries.[5,6] How-

ever, in reality, the establishment of this preventive measure is difficult, since it requires changes in dietary habits that consequently demand cultural changes and the need to make individuals realize the value of oral health, which rarely occurs.

The use of fluorides has also been widely employed in the last decades.[7-9] However, indiscriminate use of systemic fluoride can cause adverse effects, such as the occurrence of dental fluorosis.[10] Therefore, other preventive measures are needed.

Greater emphasis has been given to biofilm control as a probable preventive measure against dental caries.[11-15] Mechanical dental plaque removal may be achieved by self-control measures (toothbrushing and flossing) or by professional prophylaxis. It should be highlighted that self-control measures are subject to the child's and his/her family's limitations in performing oral hygiene. Taking this into consideration, children have been submitted to preventive programs based on regular plaque control by professional prophylaxis, which aims to compensate for poor toothbrushing.[11-16]

When regularly performed, professional prophylaxis may significantly reduce the progression of dental caries.[11-15] One method for performing this procedure is the use of sodium bicarbonate.

Investigations comparing the effectiveness of prophylaxis using sodium bicarbonate with that performed using rubber cup and pumice reveal that sodium bicarbonate removes dental plaque more effectively, especially in pit and fissure regions.[16,17] Tooth wear could be a possible adverse effect of the regular use of sodium bicarbonate. Thus, several studies have been conducted to quantify the amount of tooth structure removed in each session of professional prophylaxis using sodium bicarbonate.[18-20] In general, authors agree that sodium bicarbonate has a slight effect on intact enamel.[18-20] However, these studies evaluated the dental surface immediately after the procedure, while doubts still remain concerning the protective capacity of the oral environment due to the presence of saliva and/or fluoride. Oral fluids may offer some protection, even when considering the loss of tooth structure by abrasion caused by the mechanical action of sodium bicarbonate under water pressure.

Saliva is rich in minerals and proteins, it is supersaturated with calcium and phosphate ions, and it lubricates the teeth.[21] Thus, saliva acts against demineralization and may be able to recover the slight mineral loss of enamel caused by prophylaxis using sodium bicarbonate. Furthermore, fluoride can increase the rate of re-mineralization due to its mechanism of action.[8,22] However, it is not known whether fluoride may influence the redeposition of minerals in case of abrasion of enamel surfaces.

Considering such aspects, this *in situ* study aimed to evaluate the effect of saliva, whether associated or not with fluoride, on enamel that had previously been subjected to prophylaxis using sodium bicarbonate.

MATERIAL AND METHODS

Experimental design

This *in situ* study involved a crossover, blind design performed in two phases of 4 hours,[20] with a wash-out period of 7 days. The groups under study were: G1—treatment with sodium bicarbonate jet *in vitro* and saliva exposure for 4h *in situ*, and G2—treatment with sodium bicarbonate jet *in vitro*, 0.2% NaF rinse during the first minute and saliva exposure for 4h *in situ*. Each phase corresponded to one group: G1 or G2. Ten healthy adult volunteers living in the same fluoridated area (0.7 ppm) with a mean age of 28 years (range 23-35 years) and normal salivary flow rate took part in this study after signing an informed, written consent, approved by the IRB of Bauru Dental School, University of São Paulo. They wore acrylic palatal appliances, each containing two dental enamel blocks (Figure 1).

Preparation of the enamel blocks

Enamel blocks (4x4x3 mm) were prepared from bovine incisors sterilized by storage in 2% formaldehyde solution pH 7.0 for 30 days at room temperature.[23] The enamel surface of the blocks was ground flat with water-cooled carborundum discs (320, 600, and 1200 grits of Al2O3 papers; Buehler, Lake Bluff, IL, USA), and polished with diamond spray (1µm; Buehler).

Baseline superficial microhardness assessment

For surface enamel hardness determination, five indentations were done in different regions of the block (25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). The microhardness tester was

calibrated before use and all measurements were conducted by the same person throughout the experiment. Forty blocks that had a mean hardness of approximately 339 KHN were selected. These blocks were randomly assigned to the two groups and the baseline hardness values of the blocks were similar for both groups. Each group contained 20 enamel blocks (duplicates) or 10 experimental units (n=10). These blocks were randomly assigned to the two groups.

In vitro sodium bicarbonate jet application

To maintain reference surfaces, two layers of nail varnish were applied on half of the blocks´ surfaces.[18-20] On the other half, the sodium bicabornate jet was applied (Dabi Atlante Industrias Médico Odontológicas LTDA) at a distance of 5 mm from the block for 10 seconds, at a 90º angle, without interruption.[18-20] After this procedure, the nail varnish was removed and the enamel hardness and profilometric analyses were conducted.

Surface profilometry

Enamel wear was determined by profilometry (Hommel Tester T1000, VS, Schwenningen).[1,20-22] The tracing parameters were established at Lt: 1.5 mm and Lc: 0.25 mm, and the profilometry accuracy is 0.4 µm. Four readings were performed on each block from the reference to the exposed surface. The average wear depth by volunteer of an experimental unit was computed using 8 readings, four each of two blocks.

Superficial microhardness assessment

After the profilometric measurements were made, the blocks were once again subjected to microhardness tests in the same manner as described above. The nail varnish was reapplied on half of the blocks' surfaces.

In situ saliva and fluoride effect

Ten days prior to the beginning of the experiment, and also during the whole experimental period, the volunteers brushed their teeth with fluoridated dentifrice (Colgate® - 1450 ppm of fluoride). Custom-made acrylic palatal devices were made with 2 cavities in the surface of each appliance. One block was fixed with wax in each of the 2 cavities. In G1, the volunteers wore the appliance inside the mouth for 4h. In G2, the volunteers also wore the appliance for 4h, but during the first minute, with the appliance in place, they rinsed using a 0.2% NaF solution (Pharmácia Específica LTDA, Bauru, SP).

Profilometry measurements and superficial microhardness assessment

The nail varnish was removed and the microhardness and profilometry measurements were made as described above.

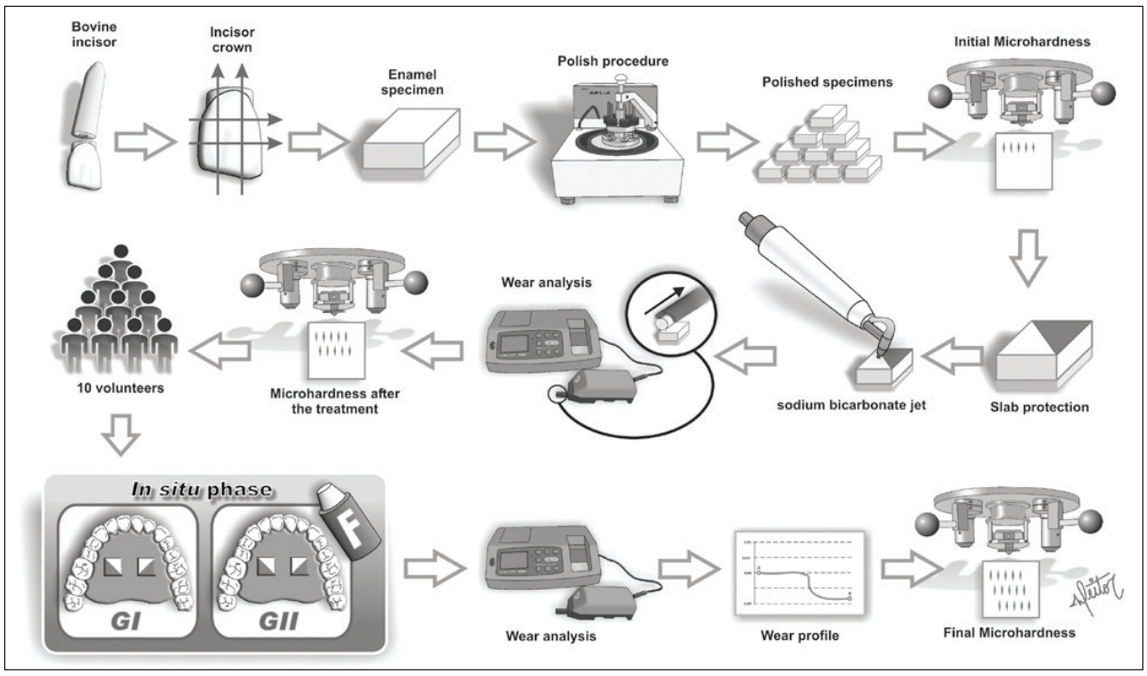

Figure 1. Schematic drawing of the experimental design.

Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked for the response variables tested. Since the assumptions were satisfied, ANOVA and Tukey´s tests were carried out for statistical comparisons for superficial enamel hardness data. A t-test was used to compare G1 and G2, taking into account the differences of final and baseline surface profiles (wear). For all tests, the significance level was set at P≤.05.

RESULTS

Concerning the variable surface hardness, statistical analysis revealed no statistically significant difference in the action of saliva compared to saliva associated with fluoride (Table 1). Professional prophylaxis applied on enamel blocks *in vitro* led to statistically significantly lower surface hardness compared to baseline hardness values and after the *in situ* stage (Table 1).

With regard to wear, there was no significant difference between wear after prophylaxis compared to the *in situ* situation (Table 1). The wear of G1 was different from that found in G2; however, this difference was also observed between groups before the *in situ* stage (Table 1). Thus, the paired t test was applied to allow a more correct analysis (P<.05), considering the results of differences in wear (profile after study in the *in situ* stage minus profile after prophylaxis), so that the initial differences would not be considered. The results reveal that the wear of blocks submitted to saliva (-0.0016) was similar to the wear of blocks submitted to saliva associated with fluoride (-0.0074) (P=0.6196).

DISCUSSION

Most investigations of the wear caused by professional methods for dental plaque removal have an *in vitro* design18-20 and do not consider the protective properties of the oral environment, mainly related to the action of saliva. The *in situ* models suggested by Kolourides et al[24] for investigations on dental caries present great advantages compared to laboratory studies. The *in situ* model may simulate intraoral events and simultaneously standardize the experimental conditions; at a short time period, they allow for the development of enamel alterations or recovery.[25]

Bovine incisors was used in this study because they have been widely used in dental research,[26-28] especially because of their structural similarity to human tooth,[29] homogeneous and reproducible surface,[30] and lower biological variability,[30] since they are not exposed to high concentrations of fluoride.[24,25,28]

The 4-hour period for duration of each step was based on the findings of Ribeiro et al.[20] These authors demonstrated that, after one hour in an *in vitro* study, saliva can recover previously abraded bovine enamel. Moreover, this period of time is the maximum mean period during which the oral cavity is not subjected to other stimuli after professional prophylaxis, such as contact with food.[20]

In the present study, after professional prophylaxis with sodium bicarbonate *in vitro*, there was a reduction in surface enamel hardness. Since this exposed surface was less mineralized, saliva had a remineralizing action, with a significant increase in surface hardness, which was not different from baseline hardness. Similar results were observed by Ribeiro et al,[20] who conducted an *in vitro* study to investigate the action of saliva on enamel previously submitted to professional prophylaxis. They observed an increase in surface hardness after one hour.

Conversely, analysis of wear did not reveal any reparative action of saliva. After the *in situ* stage, during which the blocks were submitted to the ac-

Table 1. Mean and standard deviation (sd) of surface microhardness (KHN) and wear (μm) of experimental groups, after initial selection, in the *in vitro* stage after prophylaxis and in the *in situ* stage.

Response variable	Initial selection Initial – mean±sd	*In vitro* phase Prophy – mean±sd	*In situ* phase Final – mean±sd
Microhardness G1	340.05±16.61 [a, A]	329.50±35.68 [a, B]	354.6±37.81 [a, A]
Microhardness G2	338.25±15.63 [a, A]	312.35±46.30 [a, B]	340.15±21.76 [a, A]
Wear G1	_____	0.495±0.176 [a, A]	0.493±0.181 [a, A]
Wear G2	_____	0.312±0.157 [b, A]	0.319±0.160 [b, A]

*: Values in the same column followed by different lower case letters and in the same line followed by capital letters are statistically significant (P<.05).

tion of saliva for 4 hours, there was no significant reduction in wear. On the other hand, during investigations on demineralized enamel by artificial caries solution, the authors[20] observed reduction in wear, in agreement with Honório et al.[18] These results may be explained by the fact that the enamel with white spot lesions is especially more prone to chemically receiving minerals (calcium and phosphate) from saliva, since the enamel crystals are altered and consequently have lower mineral content, which are still present in the tooth structure, thus allowing recovery and a slight gain in height.[36] Conversely, sound enamel submitted to mechanical wear completely loses the surface crystals, precluding recovery and structure gain in height, since there is no matrix for deposition of ions from the saliva.

In the present study, care was taken to assure the homogeneity of experimental groups by random division according to initial surface enamel hardness. Notwithstanding, G1 exhibited statistically higher wear than G2 after *in vitro* professional prophylaxis, which was performed equally for both groups, even though hardness values after prophylaxis were not different between the groups. There is no explanation for such an outcome; however, to overcome this problem, the difference in wear should be considered to allow actual comparison of influence of the *in situ* stage between G1 and G2.

The action of fluoride on a structure previously submitted to professional prophylaxis was investigated because no studies have addressed its effect on exclusively abraded surfaces, even though the remineralizing effect of this element on surfaces previously demineralized by dental caries is well established. The 0.2% NaF solution was selected due to the demonstrated clinical action for caries remineralization and easy utilization.[32,33] A solution was employed because utilization of dentifrice might promote further wear, since its utilization is related to toothbrushing (abrasion).

The results revealed that the action of saliva (G1) was not different from saliva associated with 0.2% sodium fluoride (G2) for both surface enamel hardness and wear variables. Both promoted an increase in surface enamel hardness (G1, 329 and 354 KHN; G2, 312 and 340 after prophylaxis and after the *in situ* stage, respectively), yet none could reestablish the enamel in height (G1 -0.0016 and G2 -0.0074 of mean difference).

Initially, it was assumed that utilization of fluoridated dentifrice before the *in situ* stage might promote a residual effect of fluoride, thus masking the effect of fluoride mouth rinse. This phenomenon hardly occurred, since care was taken to assure a one-hour interval between toothbrushing and onset of the *in situ* stage, to avoid the action of fluoride from dentifrice in the study.[34] However, Bruun, Givskov, and Thylstrup[35] found residual fluoride in saliva for more than one hour after toothbrushing with fluoridated dentifrice, compared to saliva of the placebo dentifrice. Thus, future studies should ideally evaluate the fluoride in saliva before onset of the *in situ* stage, increase the washout period of dentifrice, or employ non-fluoridated dentifrice.

The type of fluoride may also influence the outcome. High concentration fluorides, such as fluoridated varnishes, have demonstrated greater effectiveness in remineralization.[36] In addition, some authors[37,38] observed that concentrations above 500 ppm of fluoride did not produce significant increase in remineralization. Similar studies should be conducted to compare several concentrations of fluoride to promote r a better understanding of the action of fluoride on abrasion. Moreover, its action should be evaluated not only on "intact" enamel, but also on previously demineralized enamel.

Thus, considering the importance of mechanical biofilm removal as a caries preventive measure, regardless of the effectiveness of the *in situ* action of saliva associated or not with fluoride to recover the enamel structure lost during prophylaxis with sodium bicarbonate, the wear produced by this procedure was very slight. Therefore, professionals may safely perform prophylaxis without concern regarding the adverse effects of significant loss of intact tooth structure. In the case of high caries risk patients who present dental plaque accumulation, high concentration topical fluoride products may be professionally applied after prophylaxis, enhancing remineralization.

CONCLUSIONS

Based on the results, it was concluded that saliva exhibited a similar effect as saliva associated with fluoride; after 4h of *in situ* remineralization, there was no recovery in height of the tooth structure lost due to application of sodium bicarbonate on sound enamel.

ACKNOWLEDGMENTS

The authors would like to gratefully acknowledge Prof. Dr. José Roberto Pereira Lauris for the statistical analysis.

REFERENCES

1. Honório HM, Rios D, Santos CF, Magalhães AC, Buzalaf MA, Machado MA. Effects of erosive, cariogenic or combined erosive/cariogenic challenges on human enamel: an *in situ*/ex vivo study. *Caries Res* 2008;42:454-459.
2. Keyes PH. The infectious and transmissible nature of experimental dental caries. Findings and implications. *Arch Oral Biol* 1960;1:304-320.
3. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. *J Dent Res* 2004;83:C35-C38.
4. Takahashi N, Nyvad B. Caries ecology revisited: microbial dynamics and the caries process. *Caries Res* 2008;42:409-418.
5. Gustafsson BE, Quensel CE, Lanke LS, Lundqvist C, Grahnen H, Bonow BE, Krasse B. The Vipeholm dental caries study; the effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years. *Acta Odontol Scand* 1954;11:232-264.
6. Harris R. Biology of the children of hopewood house, bowral, australia. 4. Observations on dental-caries experience extending over five years (1957-61). *J Dent Res* 1963;42:1387-1399.
7. Ccahuana-Vásquez RA, Tabchoury CP, Tenuta LM, Del Bel Cury AA, Vale GC, Cury JA. Effect of frequency of sucrose exposure on dental biofilm composition and enamel demineralization in the presence of fluoride. *Caries Res* 2007;41:9-15.
8. Hellwig E, Lussi A. What is the optimum fluoride concentration needed for the remineralization process? *Caries Res* 2001;35:57-59.
9. Paes-Leme A, Dalcico R, Tabchoury C, Del Bel Cury A, Rosalen P, Cury J. *In situ* effect of frequent sucrose exposure on enamel demineralization and on plaque composition after APF application and F dentifrice use. *J Dent Res* 2004;83:71-75.
10. Fejerskov O, Manji F, Baelum V. The nature and mechanisms of dental fluorosis in man. *J Dent Res* 1990;69:692-700.
11. Axelsson P, Lindhe J, Nystrom B. On the prevention of caries and periodontal disease. Results of a 15-year longitudinal study in adults. *J Clin Periodontol* 1991;18:182-189.
12. Axelsson P, Lindhe J, Waseby J. The effect of various plaque control measures on gingivitis and caries in schoolchildren. *Community Dent Oral Epidemiol* 1976;4:232-239.
13. Axelsson P, Lindhe J. Effect of oral hygiene instruction and professional toothcleaning on caries and gingivitis in schoolchildren. *Community Dent Oral Epidemiol* 1981;9:251-255.
14. Axelsson P, Lindhe J. The effect of a preventive programme on dental plaque, gingivitis and caries in schoolchildren. Results after one and two years. *J Clin Periodontol* 1974;1:126-138.
15. Holmen L, Mejare I, Malmgren B, Thylstrup A. The effect of regular professional plaque removal on dental caries in vivo. A polarized light and scanning electron microscope study. *Caries Res* 1988;22:250-256.
16. Strand GV, Raadal M. The efficiency of cleaning fissures with an air-polishing instrument. *Acta Odontol Scand* 1988;46:113-117.
17. Weaks LM, Lescher NB, Barnes CM, Holroyd SV. Clinical evaluation of the Prophy-Jet as an instrument for routine removal of tooth stain and plaque. *J Periodontol* 1984;55:486-488.
18. Honório HM, Rios D, Abdo RCC, Machado MAAM. Effect of different prophylaxis methods on sound and demineralized enamel. *J Appl Oral Sci* 2006;14:117-123.
19. Hosoya Y, Johnston JW. Evaluation of various cleaning and polishing methods on primary enamel. *J Pedod* 1989;13:253-269.
20. Ribeiro HZV, Lima JEO, Vono BG, Machado MAAM, Silva SMB. Airpolishing effect on bovine enamel and the posterior remineralizing effect of saliva. An *in vitro* study. *J Appl Oral Sci* 2006;14:193-197.
21. Mandel ID. The functions of saliva. *J Dent Res* 1987;66:623-627.
22. Llena C, Forner L, Baca P. Anticariogenicity of casein phosphopeptide-amorphous calcium phosphate: a review of the literature. *J Contemp Dent Pract* 2009;10:1-9.
23. Magalhães AC, Rios D, Honório HM, Jorge AM Jr, Delbem AC, Buzalaf MA. Effect of 4% titanium tetrafluoride solution on dental erosion by a soft drink: an *in situ*/ex vivo study. *Arch Oral Biol* 2008;53:399-404.
24. Koulourides T, Chien MC. The ICT *in situ* experimental model in dental research. *J Dent Res* 1992;71:822-827.
25. Zero DT. *In situ* caries models. *Adv Dent Res* 1995;9:214-230.
26. Amaechi BT, Higham SM, Edgar WM. Factors affecting the development of carious lesions in bovine teeth *in vitro*. *Arch Oral Biol* 1998;43:619-628.
27. Featherstone JD, Mellberg JR. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. *Caries Res* 1981;15:109-114.

28. Mellberg JR. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity *in situ*. *J Dent Res* 1992;71:913-919.
29. Sato I, Sunohara M, Mikami A, Yoshida S, Sato T. Comparison between deciduous and permanent incisor teeth in morphology of bovine enamel. *Okajimas Folia Anat Jpn* 1999;76:131-135.
30. Manning RH, Edgar WM. Intra-oral models for studying de- and remineralization in man: methodology and measurement. *J Dent Res* 1992;71:895-900.
31. Arends J, Christoffersen J. Nature and role of loosely bound fluoride in dental caries. *J Dent Res* 1990;69:601-605.
32. Ogaard B, Rolla G, Dijkman T, Ruben J, Arends J. Effect of fluoride mouthrinsing on caries lesion development in shark enamel: an *in situ* caries model study. *Scand J Dent Res* 1991;99:372-377.
33. Ogaard B, Rolla G, Helgeland K. Uptake and retention of alkali-solube and alkali-insoluble fluoride in sound enamel in vivo after mouthrinses with 0.05% or 0.2% NaF. *Caries Res* 1983;17:520-524.
34. Bruun C, Lambrou D, Larsen MJ, Fejerskov O, Thylstrup A. Fluoride in mixed human saliva after different topical fluoride treatments and possible relation to caries inhibition. *Community Dent Oral Epidemiol* 1982;10:124-129.
35. Bruun C, Givskov H, Thylstrup A. Whole saliva fluoride after toothbrushing with NaF and MFP dentifrice with different F concentrations. *Caries Res* 1984;18:282-288.
36. Eakle WS, Featherstone JD, Weintraub JA, Shain SG, Gansky SA. Salivary fluoride levels following application of fluoride varnish or fluoride rinse. *Community Dent Oral Epidemiol* 2004;32:462-469.
37. Cruz R, Ogaard B, Rolla G. Uptake of KOH-soluble and KOH-insoluble fluoride in sound human enamel after topical application of a fluoride varnish (Duraphat) or a neutral 2% NaF solution *in vitro*. *Scand J Dent Res* 1992;100:154-158.
38. Damato FA, Strang R, Stephen KW. Effect of fluoride concentration on remineralization of carious enamel: an *in vitro* ph-cycling study. *Caries Res* 1990;24:174-180.