The erosive effects of saliva following chewing gum on enamel and dentine: an *ex vivo* study

E. M. Paice,[1] R. W. Vowles,[2] N. X. West[3] and S. M. Hooper[4]

IN BRIEF

- Alerts readers to the fact that chewing acidic gums can pose a potential risk of enamel and/or dentine erosion, particularly when individuals repeatedly refresh with new pieces of gum.
- Highlights relationships of enamel and dentine erosion to saliva buffering and tooth brushing.
- The study confirms that non-acidic gum causes no detrimental effects with respect to enamel and dentine erosion.

RESEARCH

Objectives The primary objective was to determine the erosive effect of expectorated saliva, following chewing acidic gum, on enamel and dentine samples, using a non-acidic gum as a negative control. Secondary objectives were to determine the effect of brushing enamel and dentine samples and the effect of individual saliva pH and buffering. **Design** A single-centre, single-blind, placebo-controlled, two-way crossover study. **Setting** A clinical trial, involving healthy participants, undertaken at Bristol Dental School and Hospital. **Methods** Eight healthy participants expectorated saliva onto prepared enamel and dentine samples while chewing gum (strawberry flavoured acidic gum [active] or peppermint flavoured non-acidic gum [control]). Half of the enamel and dentine samples were brushed before measurement by contact profilometry. **Main outcome measures** Mean enamel and dentine erosion, with and without brushing and the relationship to salivary buffering. **Results** At 10 days, mean depth of surface loss from dentine samples (95% CI), following chewing of acid-containing gum and subsequent brushing, was -11.34 μm (2.22 μm) and from un-brushed dentine samples was -11.02 μm (1.71 μm). No significant erosion was noted for other groups. **Conclusions** Frequent chewers of acid-containing gums are susceptible to dentine erosion even in the presence of good salivary buffering. Enamel erosion was insignificant within the time constraints of the present study but warrants further investigation.

INTRODUCTION

A leading chewing gum manufacturer in the UK has stated that the British currently chew their way through £293 million worth of chewing gum per year, an increase of 29% on 2001 figures.[1] Chewing gum is an increasingly popular habit and has for some years been promoted by the dental profession as being beneficial for the dentition in reducing the risk of caries,[2] and improving salivary flow.[3] Medical and dental researchers have considered the benefit of chewing gum as a means to deliver therapeutic agents: for example, calcium carbonate to alleviate heartburn and gastro-oesophageal reflux,[4] chlorhexidine to reduce plaque deposition[5] and casein phosphopeptide-amorphous calcium phosphate to remineralise enamel subsurface lesions.[6] The potential benefits have been extensively investigated and reviewed[7] but literature is lacking in reporting potential detrimental effects of acidic chewing gums in relation to tooth surface loss.

Enamel and dentine erosion in relation to acid foods and beverages, and attempts to reduce such erosion by modification of constituents, is well documented,[8,9] but research to reduce erosion has not extended to acidic chewing gum. Toothbrushing may exacerbate erosion by abrasion, another process contributing to tooth wear and these processes can be synergistic. Investigations have taken place with respect to combined effects in relation to beverages but not in relation to acidic chewing gum.[10,11] The act of chewing gum also has the potential to introduce a degree of tooth wear attributable to attrition.

Any suggestion implicating chewing gum as a causative agent of detrimental effects on the dentition warrants further investigation. Interest in the acidity of certain chewing gums and the potential for dental erosion was generated by observation of tooth wear, characteristics of acid erosion, and preferential tooth surface loss affecting the occlusal surfaces of all posterior teeth in a patient admitting to frequent use of flavoured acid chewing gum but with no other relevant medical history or report of gastric erosion.

The primary objective of this paper was to determine the erosive effect of expectorated saliva from human participants following chewing acidic based chewing gum compared to a non-acidic based chewing gum, on enamel and dentine samples. Surface loss was measured by contact profilometry (SF200 surfometer, Planer Products Ltd., Windmill Road, Sunbury-on-Thames, Middlesex TW16 7HD). Secondary objectives were to determine the effect of tooth brushing the enamel and dentine samples immediately following exposure to expectorated saliva following acidic and non-acidic chewing gum and to determine the effect of saliva pH and salivary buffering.

MATERIALS AND METHODS

Design

The trial design was a single-centre, single-blind, randomised two-way crossover study, involving healthy participants, undertaken

[1]Senior House Officer, [2]Dental Materials Support Officer, [3]Professor in Restorative Dentistry, [4*]Consultant Senior Lecturer, University of Bristol Dental Hospital, Lower Maudlin Street, Bristol, BS1 2LY
*Correspondence to: Miss Susan Hooper
Email: susan.hooper@bristol.ac.uk
Tel: +44 (0)117 342 4316

Refereed Paper
Accepted 30 September 2010
DOI: 10.1038/sj.bdj.2010.51
©British Dental Journal 2010; 210: E3

to compare the erosive effect of expectorated saliva following chewing acidic and non-acidic gums on human enamel and dentine specimens.

Subjects

A sample size of eight healthy participants was chosen based on experience from a previous study.[12] All participants worked at Bristol Dental Hospital and were 18 years old or older. Inclusion and exclusion criteria for acceptance of subjects in the study were as described and used in previous publications.[9,13,14] They were all subjects with no medical or pharmaco-therapeutic history, such as acid reflux, which might influence the conduct or outcome of the study. They all underwent a dental screening examination and were caries-free, with good gingival health and no clinical signs or symptoms of pathological tooth surface loss. Ethical approval for the study was granted by the United Bristol Healthcare NHS Trust Ethics Committee. The study was conducted according to the Guidelines for Good Clinical Practice (ICHP) and all data were anonymised for analysis. Subjects received verbal and written information on the study, and gave signed and witnessed consent to participate. The study was blinded to the person responsible for performing the surface profilometry measurements of erosion. An individual not otherwise involved in the study monitored the conduct of the study and the case record forms.

Methods

Two gums, both market sales leaders, were selected for use in the study: Seriously Strawberry flavoured Hubba Bubba (Wrigley Company, Plymouth, UK) and Extra Peppermint (Wrigley Company, Plymouth, UK). The mean pH (SD) of five samples of Seriously Strawberry was 2.84 (0.04) and that of Extra Peppermint was 6.71 (0.11). The pH values of these gums are representative of six popular sugar gums (pH range 2.70–3.56) and seven sugar-free gums (pH range 6.58-7.57) investigated for this study.

The enamel and dentine specimens were derived from surgically removed human third molars from 18-year-olds or older individuals of either gender. Sterilisation of the teeth was achieved by soaking in hypochlorite solution for 24 hours with 20,000 ppm available free chlorine. Enamel and dentine specimens were embedded in epoxy resin (Stycast 1266, Emmerson and Cumming Specialist Polymers, Belgium) and polished to produce a flattened window, the specimens having dimensions of 8 mm x 5 mm x 2 mm. Specimens were baselined by contact profilometry. The head unit of the profilometer traversed the specimen at a constant velocity from left to right at a speed of 10 mm/min. The measuring head was fitted with a diamond stylus of 20 μm tip radius. The force of the stylus on the surface varied linearly with deflection at the rate of 8 mg force per micron deflection, the maximum force at 100 mm being 1.0 g. To be accepted into the study the baseline readings for enamel samples needed to achieve surface profiles of ± 0.1 mm, and dentine samples ± 0.3 mm. Polyvinyl chloride (PVC) tape was used on the enamel and dentine samples to delineate a zone approximately 1.5 mm in width for measuring surface loss. The samples were designated by coloured tape to identify whether they were to be brushed or left unbrushed. Each enamel and dentine sample was identified with a unique number on the back using a permanent pen and assigned to a specific subject participating in the study. Two perspex strips, each with four mounted samples (two enamel and two dentine), were placed on the base of a small container in readiness for collection of saliva expectorant by a specified subject. One perspex strip held the samples for brushing and the other the samples to be withheld from brushing.

Before each attendance subjects were asked to abstain from drinking or eating for one hour. They attended four times a day for ten successive study days for each gum according to study randomisation and were supervised throughout each attendance. Subjects attended at the same times each day: 9.00 am, 11.00 am, 1.00 pm and 3.00 pm (± 30 minutes for each of these appointments). Each time subjects attended the Clinical Trials Unit they confirmed they had abstained from drinking or eating during the previous hour and they then chewed three pieces of gum consecutively, each one for four minutes (total 12 minutes). They spat any saliva generated at 30-second intervals onto the enamel and dentine samples held in a plastic container. Subjects gently agitated the container to ensure saliva remained on the exposed enamel and dentine windows of each sample. After four minutes the samples were transferred to a new container and the subject commenced chewing a fresh piece of gum. This process was repeated for the final piece of gum. On completion of each 12-minute chewing cycle, the samples were rinsed with de-ionised water.

After each visit, site personnel dry-brushed the two designated enamel and two designated dentine samples for each subject with a powered toothbrush (Braun Oral B advanced power 900 series fitted with an EB17 Flexisoft head; Gillette UK, Middlesex) for 30 seconds. The brushed and unbrushed samples were then placed in a subject-designated container with cotton wool rolls dampened with Volvic® water.

At the start and end of each treatment day, plus before and after taking the profilometry measurements, the enamel and dentine samples were disinfected by dipping in 0.2% w/v chlorhexidine gluconate, Corsodyl® mouthrinse (GlaxoSmithKline, Brentford, UK) for three minutes. The samples were then rinsed in 200 mls of mineral water, Volvic® (Danone Waters, London, UK), in a 500 ml beaker.

On days 5 and 10 all enamel and dentine samples, brushed and unbrushed, were examined by profilometry for surface loss by a trained and experienced profilometry operator.[11,12,15–17] Each time the profilometer was turned on for a measuring session, a flat enamel calibration sample was measured to ensure two consecutive readings were within ± 0.1 μm. The surface profiles of the enamel and dentine samples pre- and post-treatment identified the amount of tooth surface lost in microns. All samples were read twice and an average reading was used for analysis.

Salivary buffering capacity and change in pH during chewing

Each subject was also asked to attend on two separate occasions for the purpose of investigating the buffering capacity of their saliva and its change in pH during chewing the test products. All volunteers attended at 9.00 am (± 30 minutes) consistent with the first daily attendance for the main study investigating the erosive effects of saliva following chewing gum on enamel and dentine and subsequent brushing. The buffering capacity for each subject was established by measuring the pH of a freshly supplied 3 ml unstimulated saliva sample using a calibrated glass-bodied combination pH reference electrode (Jenway, Dunmow, UK) and re-measuring

Table 1 Summary of enamel and dentine loss at days 5 and 10 post-exposure to saliva following chewing gum

Sample	Day	Test group	Mean depth (μm) N = 8 for HB, N = 7 for EP	95% confidence interval (μm)
Enamel	5	HB brushed	-0.11	0.13
Enamel	5	HB unbrushed	-0.15	0.17
Enamel	5	EP brushed	-0.02	0.05
Enamel	5	EP unbrushed	-0.02	0.04
Enamel	10	HB brushed	-0.33	0.49
Enamel	10	HB unbrushed	-0.16	0.18
Enamel	10	EP brushed	-0.03	0.03
Enamel	10	EP unbrushed	-0.03	0.04
Dentine	5	HB brushed	-6.88	1.33
Dentine	5	HB unbrushed	-6.74	1.30
Dentine	5	EP brushed	-0.10	0.12
Dentine	5	EP unbrushed	0.02	0.19
Dentine	10	HB brushed	-11.34	2.22
Dentine	10	HB unbrushed	-11.02	1.71
Dentine	10	EP brushed	-0.06	0.13
Dentine	10	EP unbrushed	0.02	0.15

HB: Seriously Strawberry Hubba Bubba; EP: Extra Peppermint; Subject 2 excluded from Extra Peppermint leg

the pH after additions of 200 μl 0.05M hydrochloric acid (HCl) until 2000 μl HCl had been added in total.

The change in pH during chewing the test products was measured by first measuring the pH of a freshly supplied 3 ml unstimulated saliva sample from each subject. Subjects were then asked to chew a single piece of one of the test formulations for 30 minutes. During chewing, subjects were asked to spit (when they would normally swallow) all saliva into sequential small bottles as per the following regimen: during the first minute into bottle 1, during the following two minutes into bottle 2, during the next two minutes into bottle 3. During the remaining 25 minutes they were asked to spit into bottles 4–8, moving to a new bottle every five minutes. This regimen was devised as more saliva is generated during the first five minutes of chewing gum. The pH was recorded twice for each sample and an average reading used for analysis.

Statistics

A paired t-test was used to compare the two gums selected for the study. Further analysis on the erosion was undertaken using the Kruskal-Wallis Test. Mean erosion, at the 95% confidence level, was established for each participant and ranked for comparison with salivary pH and buffering.

Outcome measures

The mean enamel and dentine tooth surface loss, with and without subsequent brushing, for individual subjects, and the relationship of erosion to salivary pH and buffering.

RESULTS

Eight healthy participants, five females and three males, completed the study having a mean age of 36.5 years (range 25-63.5 years).

The differences between the baseline readings and the readings taken at day 5 and day 10 were calculated for each participant and then averaged to provide a mean erosion figure with a 95% confidence interval (CI) for each group of samples. Samples were grouped for day 5 and day 10 readings by

1. type of gum chewed, Hubba Bubba or Extra Peppermint
2. enamel or dentine sample
3. brushed or unbrushed sample (see Table 1).

Each participant's dentine samples were shown to have undergone considerable erosion after exposure to saliva having chewed Seriously Strawberry Hubba Bubba chewing gum. After five days and ten days brushed dentine samples demonstrated greatest surface loss at -6.88 μms (CI 1.32 μms) and -11.34 μms (CI 2.22 μms). There was strong statistical evidence (Kruskal-Wallis) to demonstrate a difference ($p < 0.0001$) between all Hubba Bubba and Extra Peppermint groups subjected to the same regimens.

When brushed and unbrushed samples in otherwise similar groups were compared statistical evidence only identified a significant difference ($p < 0.05$) after 10 days, between brushed and un-brushed dentine samples exposed to Extra Peppermint. In these groups the surface loss was minimal, between -0.10 μms (CI 0.12 μms) and +0.2 μms (CI 0.19 μms) taking consideration of readings at both day 5 and day 10 respectively. Statistical evidence did not identify significant differences between any other groups when comparing brushed and unbrushed samples.

Salivary buffering capacity and change in pH during chewing gum

Combining erosion results for brushed and unbrushed samples, correlation coefficients between rankings for tooth surface loss by subject and amount of acid required to decrease the pH by one unit were good for Hubba Bubba enamel (0.93) and satisfactory for dentine (0.64) but were poor for Extra Peppermint enamel (0.20) and dentine (0.25) respectively.

The amount of 0.05M hydrochloric acid (HCl) added to a 3 ml unstimulated saliva sample from each subject to lower the pH by one unit was used to rank the salivary buffering capacity of each subject. Subjects 1 and 2 required the least HCl addition (0.04 ml, 0.08 ml respectively) while subjects 4 and 5 required the greatest (0.24 ml, 0.37 ml respectively) [see Table 2 for ranking]. Subjects 4 and 5 also required the most HCl addition (0.4 ml and 0.5 ml respectively) to reduce the pH of the saliva samples below the critical pH of 5.5.

The lowest recorded pH following chewing acidic gum for each subject ranged from 3.52 to 4.20. Figures 1 and 2 illustrate the speed of neutralisation of the saliva after chewing Extra Peppermint and Seriously Strawberry Hubba Bubba respectively. After chewing Seriously Strawberry Hubba

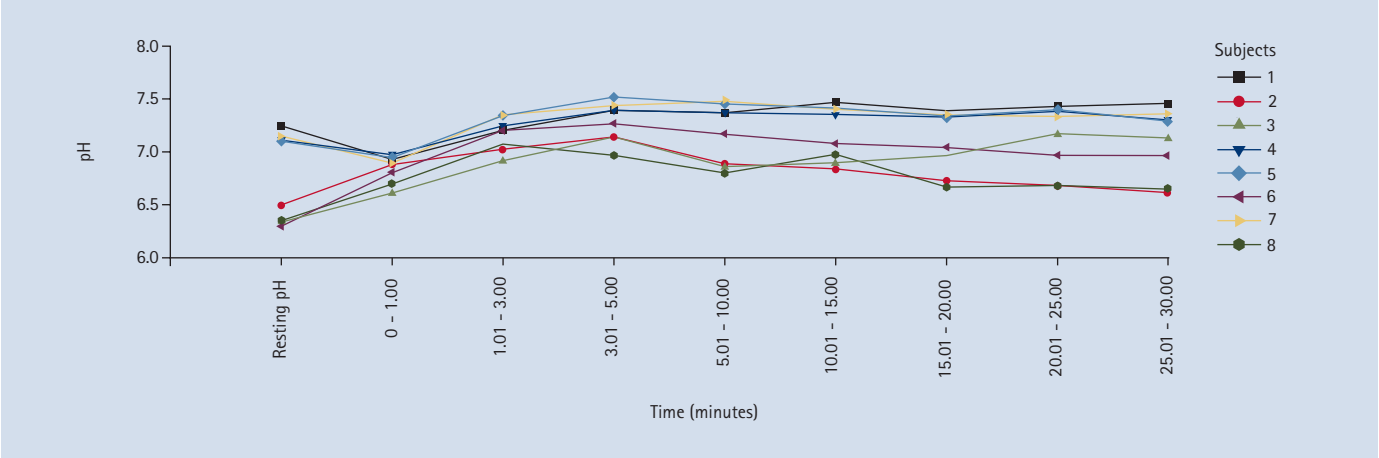

Fig. 1 Speed of neutralisation of the oral cavity after chewing Extra Peppermint gum

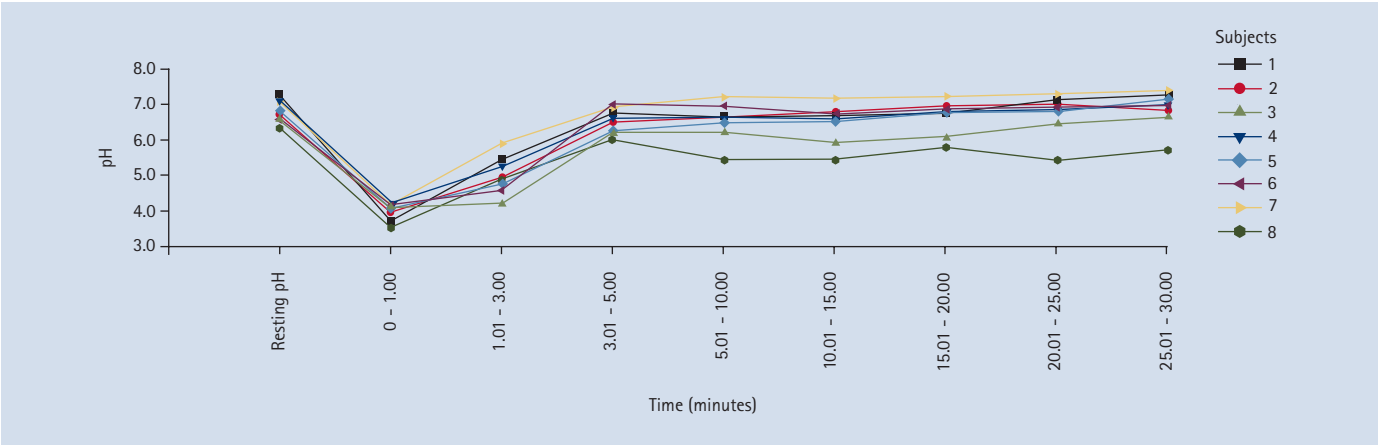

Fig. 2 Speed of neutralisation of the oral cavity after chewing Seriously Strawberry Hubba Bubba gum

Bubba for one minute the mean salivary pH of all subjects was 3.98, whereas for Extra Peppermint the mean salivary pH of all subjects was 6.80. However, the recovery in pH of saliva while chewing Seriously Strawberry Hubba Bubba was rapid; after three minutes the mean pH was 4.99 (Extra Peppermint pH 7.02) and after five minutes the mean pH was 6.53, a level approaching 7.27, the mean salivary pH of subjects chewing Extra Peppermint.

Subject ranking was also undertaken with respect to time taken for saliva pH to return to resting levels (range 3 mins–30 mins) although within 5 mins all subjects had a saliva pH above the critical pH of 5.5 (pH range 5.99–7.00). Only one subject had a pH above the critical pH after 3 minutes.

Table 2 Comparisons of participant ranking for dentine loss following chewing Seriously Strawberry Hubba Bubba, salivary buffering and salivary pH recovery after chewing gum

Dentine erosion (brushed and unbrushed): most to least erosion	Salivary titration drop in pH by 1 unit: least to most HCl added	Lowest pH recorded following chewing: lowest to highest pH	Time taken for saliva to return to resting pH: slowest to quickest time
1	1	8	4,8
2	2	1	
4	8	6	1,3
8	6	2	
6	3	5	5
5	7	3	2
3	4	4	7
7	5	7	6

HCl=0.05M hydrochloric acid

DISCUSSION

Erosive tooth wear is an increasingly important factor when considering the long-term health of the dentition and it is well established that the aetiology is multifactorial[18,19]. Chewing gum adds a behavioural component which, with acidic gums, can compound erosive tooth surface loss by introducing a component of attrition. In the presence of saliva, chemical and biological issues further compound identification of the causative factors. This study sought to examine, *ex vivo*, the interaction of these latter effects. Chewing gum for 20 minutes has been shown to increase both salivary flow rate and salivary pH and has been deemed beneficial to oral and dental health with a potential to promote enamel remineralisation.[5,20] There is sparse evidence in the literature to suggest any chewing gums contribute to enamel or dentine erosion although some evidence has related to the potential for plaque pH to remain low

potentially affecting caries susceptibility. Low plaque pH levels occur during and after the chewing of sucrose (acidic)-containing chewing gum, despite the masticatory stimulation of saliva.[5,21] Although the present study indicated a rapid rise in salivary pH after chewing Seriously Strawberry Hubba Bubba, repeated chewing with frequent refreshment of new gum could potentially be very damaging. Subjects participating in the study stated that the flavour from the Seriously Strawberry Hubba Bubba rapidly diminished within minutes of chewing. Individuals enjoying these flavoured gums are more likely to habitually replace gum.

The pH of the Extra Peppermint gum in solution was 6.71. As this value sits above the critical pH, no enamel or dentine erosion was expected. The main artificial sweetener found in Wrigley's Extra Peppermint is xylitol, which is non-acidogenic.[21] Xylitol is not fermented to form acids at the rate recorded for conventional mono- and disaccharides for example, glucose (present in Seriously Strawberry Hubba Bubba) and sucrose which is also found in many other sugar-containing (acidic) chewing gums.[2] In this study, little or no fall in salivary pH resulted when Extra Peppermint gum was chewed (see Figure 1) when compared with chewing Seriously Strawberry Hubba Bubba (see Figure 2). The pH of the Seriously Strawberry Hubba Bubba gum in solution was 2.84. However, despite a rapid fall in pH of expectorated saliva within one minute of chewing Seriously Strawberry Hubba Bubba, this gum similarly increased salivary flow rate. Refreshing gum, by regularly chewing a new piece, is potentially very damaging. In this study, replacement of gum every four minutes resulted in marked dentine erosion. The time constraints of the present study are probably responsible for the limited enamel erosion seen. The duration of contact has been shown to be of importance.[22] If the strengths and frequencies of acidic challenges are such that the process of repair is unable to take place then erosion of tooth tissue is expected to occur.

The present study demonstrated no clinical differences between the amounts of tooth surface loss seen on the unbrushed samples compared with the brushed samples. This was a surprising result but may be attributable to the brushing taking place dry. Previous work has stated that a toothbrush alone (that is, without paste) is unlikely to cause significant abrasion to dentine.[23] A more clinically relevant approach may have been to have brushed with toothpaste. There is discussion in the literature with respect to the effect of brushing eroded surfaces. An increased susceptibility to erosion with brushing has been shown,[24] while other studies do not support the notion that brushing increases substance loss of eroded dentine.[25,26] When comparisons are made with respect to the use of fluoride or non-fluoride pastes while brushing eroded tooth surfaces, the non-fluoride paste was favourable.[27,28]

The individual saliva buffering identified that the subjects requiring the least amounts of acid to reach the critical pH were relatively consistent with ranking of their dentine erosion occurring after chewing Seriously Strawberry Hubba Bubba with the exception of one subject [subject 4] (Table 2). There was less consistency when comparing the dentine erosion ranking of the subjects to the time taken for saliva to return to resting pH after chewing Seriously Strawberry Hubba Bubba or the lowest pH recorded while chewing.

Enamel erosion was insignificant with Seriously Strawberry Hubba Bubba within the time constraints of the present study. Further studies are warranted to examine this more fully in the light of the popularity of the gums with adolescents and young adults.

CONCLUSIONS

The study concluded that Seriously Strawberry Hubba Bubba contributed to significant erosion of human dentine (mean -11.34 μm brushed, -11.02 μm unbrushed) when chewed for short periods of time with frequent intake of new pieces of gum, even in the presence of good salivary buffering.

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