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Remineralization of enamel subsurface lesions by chewing gum with added calcium

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ABSTRACT

Objectives: Chewing sugar-free gum has been shown to promote enamel remineralization. Manufacturers are now adding calcium to the gum in an approach to further promote enamel remineralization. The aim of this study was to compare the remineralization efficacy of four sugar-free chewing gums, two containing added calcium, utilizing a double-blind, randomized, crossover *in situ* model.

Methods: The sugar-free gums were: Trident Xtra Care, Orbit Professional, Orbit and Extra. Ten subjects wore removable palatal appliances with four human-enamel half-slab insets containing subsurface demineralized lesions. For four times a day for 14 consecutive days subjects chewed one of the chewing gums for 20 min. After each treatment the enamel slabs were removed, paired with their respective demineralized control slabs, embedded, sectioned and mineral level determined by microradiography. After 1-week rest the subjects chewed another of the four gums and this was repeated until each subject had used the four gum products.

Results: Chewing with Trident Xtra Care resulted in significantly higher remineralization ($20.67 \pm 1.05\%$) than chewing with Orbit Professional ($12.43 \pm 0.64\%$), Orbit ($9.27 \pm 0.59\%$) or Extra ($9.32 \pm 0.35\%$). The form of added calcium in Trident Xtra Care was CPP–ACP and that in Orbit Professional calcium carbonate with added citric acid/citrate for increased calcium solubility.

Conclusions: Although saliva analysis confirmed release of the citrate and calcium from the Orbit Professional gum the released calcium did not result in increased enamel remineralization over the normal sugar-free gums. These results highlight the importance of calcium ion bioavailability in the remineralization of enamel subsurface lesions *in situ*.

1. Introduction

The anticariogenic effect of sugar-free chewing gum has been demonstrated in a number of caries clinical trials.[1–7] The reduction in caries experience has been attributed to the stimulation of saliva increasing the available calcium and phosphate ion concentrations, limiting enamel demineralization and promoting remineralization of early caries lesions.[8,9] Recently gum manufacturers have been adding calcium in various forms to enhance the potential anticariogenic action of the gum.[10] The normal forms of calcium added to gum, calcium carbonate and/or calcium phosphate have limited solubility in saliva and are not released from the gum bolus when chewed in high enough concentrations or in a bioavailable form to affect the caries process.[10]

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One form of soluble, bioavailable calcium that has been added to sugar-free gum is casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) [Recaldent™ CASRN 691364-49-5]. The calcium in CPP–ACP is present as bioavailable ions together with phosphate ions stabilized by casein phosphopeptides containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- as water soluble nanocomplexes.[11] The CPP–ACP in sugar-free gum has been shown to remineralize enamel subsurface lesions *in situ* and to reduce the progression of approximal lesions in a randomized, controlled caries clinical trial[10,12–14]

Recently, Hass and Greenberg[15] have proposed adding food acids (e.g. citric acid/citrate) to sugar-free gum containing insoluble calcium phosphate/calcium carbonate in an approach to increase the solubility and hence bioavailability of the added calcium. However, to our knowledge these sugar-free gum formulations containing calcium phosphate/calcium carbonate together with food acids have not been tested for their ability to remineralize enamel subsurface lesions *in situ* even though they have been shown to increase the release of calcium from the gum.[15] The aim of this study was to compare the ability of four commercially available sugar-free chewing gums, two containing added calcium, to remineralize enamel subsurface lesions in an *in situ* model. One of the gums contained added calcium as CPP–ACP (Trident Xtra Care), and the other as calcium carbonate with added citric acid/citrate (Orbit Professional). The other two gums (Orbit and Extra) were normal sugar-free chewing gums.

2. Materials and methods

2.1. Study design and subject recruitment

The study was a double-blind, randomized, crossover *in situ* design with four treatments. Approval for the study was obtained from The University of Melbourne Human Research Ethics Committee and the study was conducted in accordance with Australia's National Statement on Ethical Conduct in Human Research (2007). After providing informed consent 10 healthy adult subjects (seven males and three females) were recruited from the staff and postgraduate students (age 25–55 years) of the Melbourne Dental School. All subjects had at least 22 natural teeth with no current caries activity, periodontal disease or other oral pathology. None of the subjects were using antibiotics or medications, which could have affected saliva flow rate. For each subject, the unstimulated saliva flow rate was higher than 0.2 ml/min, and the stimulated saliva flow rate, measured whilst chewing two pellets of sugar-free gum, was >1.0 ml/min.

Subjects were sequentially allocated a subject ID by the examiner on recruitment. Each subject ID had a corresponding product allocation sequence that had been generated by a biostatistician using standard randomization tables for crossover studies. Subjects and those personnel dispensing, supervising or performing analysis did not have access to the treatment codes and were unaware of which treatment had been administered. The code was not released by the sponsor until data collection and analysis were complete. Subjects were monitored for adverse events using health update questionnaires during each treatment and at the completion of the study.

2.2. Intraoral appliances

Removable mid-palatal acrylic appliances covering the first premolars to the last tooth in the arch, were fabricated for each subject as described previously.[14] The appliances were retained by four stainless steel circumferential clasps and each had two bilateral troughs to house four enamel slabs as previously described.[16]

2.3. Preparation of enamel subsurface lesions

Extracted human third molars were obtained from the Royal Dental Hospital of Melbourne. The outer enamel surface was polished wet to a mirror finish using Softex™ disks (3M, St. Paul, MN, USA) on a slow-speed contra-angle dental handpiece. Each polished surface was then sawn from the tooth as a 10 mm × 4 mm slab and the whole slab covered with acid-resistant nail varnish except for two (occlusal and gingival) mesiodistal windows (9 mm × 1 mm) separated from each other by 1 mm of varnish. Subsurface lesions were created using a demineralization buffer containing 20 g/l Carbopol 907 (BF Goodrich, Cleveland, OH, USA), 500 mg/l hydroxyapatite (Bio-Rad, Richmond, CA, USA) and 0.1 mol/l lactic acid, pH 4.8 for 96 h at 37 °C as described previously.[16] After demineralization each enamel slab was sawn through the midline of each window into two 4 mm × 4 mm half-slabs and the cut surface of each half-slab covered with nail varnish. One half-slab of each pair was retained as the demineralization control. The other enamel half-slab of the pair was inset into an intraoral appliance and retained using dental wax for the remineralization protocol. Care was taken to keep the windows free of wax. Four enamel half-slabs were inset into each appliance, two on each side in bilateral troughs.[14] The enamel slabs were recessed into the bilateral trays to produce a 1 mm space above the enamel surface to allow plaque to establish and be retained.[16]

2.4. Study protocol

The study utilized a double-blind, randomized, crossover protocol as previously described[16] with four sugar-free gums (Table 1). Two of the gums were normal sugar-free chewing gums (Orbit and Extra) and the other two had added calcium with Trident Xtra Care containing CPP–ACP and Orbit Professional containing calcium carbonate/citrate. The chewing gums were provided as coded products by the Clinical and Consumer Group, Cadbury-Adams Worldwide Research and Development (NJ, USA) in sealed packages and were stored at room temperature. The code was not released until all remineralization data had been acquired.

One of the chewing gum products was randomly assigned to each of the subjects for each of four 2-week treatment periods. Four times a day at specified times (10.00 am, 11.30 am, 2.00 pm and 3.30 pm) subjects with appliances inserted chewed two pellets or one slab of gum for 20 min. This was repeated for 14 consecutive days (treatment period). At the end of each treatment period, subjects rested for 7 days

Table 1 – Details of sugar-free chewing gum used for the study.

Gum	Type	Colour	Dose	Weight/dose (g)	Ingredients
Trident Xtra Care™ (with Recaldent™)	Slab	Blue	1 slab	1.96 ± 0.02[a]	Sorbitol, gum base, xylitol, glycerine, mannitol, natural and artificial flavours, acesulfame K, aspartame, BHT, blue 1 lake, calcium casein peptone calcium phosphate, soy lecithin, titanium dioxide and yellow 5 lake.
Orbit Professional (with calcium)	Pellet	White	2 pellets	3.13 ± 0.08	Xylitol, sorbitol, maltitol syrup, mannitol, aspartame, acesulfame-K, gum base, calcium carbonate, bulk agent dicalcium phosphate, thickening agent gum Arabic, flavour, humectant agent glycerol, colour E171 (titanium dioxide), emulsifier soy lecithin, glazing agent, carnauba wax, antioxidant BHA, colour E133 (FD&C blue 1).
Orbit	Slab	White	1 slab	1.96 ± 0.02	Sorbitol, gum base, glycerol, mannitol, natural and artificial flavours; less than 2% of: xylitol, acesulfame K, aspartame, soy lecithin, BHT (to maintain freshness). phenylketonurics; contains phenylalanine.
Extra	Slab	White	1 slab	2.69 ± 0.05	Sorbitol, gum base, glycerol, mannitol, natural and artificial flavours, hydrogenated starch hydrolysate, aspartame, acesulfame K, soy lecithin, BHT (to maintain freshness).

[a] Mean ± SD.

during which time new demineralized enamel slabs were inserted into the appliances. Subjects then crossed over to another gum product. This was repeated until each subject used each of the four products. Subjects were instructed to remove the intraoral appliances when they ate or drank and when they conducted their normal oral hygiene procedures. The times of wearing the appliances and gum chewing were recorded in a diary, collected together with used gum wrappers after each treatment period. On removing the appliances, subjects were instructed to rinse them briefly with distilled/deionised water and then to store them in a sealed, humidified containers at 37 °C until re-insertion. Subjects were also instructed to clean the appliances, avoiding the enamel slabs, with a toothbrush and fluoride-free toothpaste provided by the sponsor. Subjects maintained normal oral hygiene and dietary habits throughout the study except during the times they wore the appliances when they were instructed not to eat or drink. At the commencement of the study all subjects were provided with standard 1000 ppm fluoride toothpaste, which they used morning and night for the duration of the study. The appliances were inserted 30 min after oral hygiene procedures. The subjects lived in metropolitan Melbourne where the reticulated water supply is fluoridated at 1 ppm. After completion of each treatment period, the enamel slabs were removed from the appliances, rinsed with distilled/deionised water and stored in moist, labeled microcentrifuge tubes for further processing.

2.5. *Sectioning, microradiography and microdensitometry*

After each treatment, the enamel half-slabs were paired with their respective control half-slabs and dehydrated in absolute alcohol. Each pair of half-slabs was embedded in freshly poured transparent cold curing methacrylate resin (Paladur, Heraeus Kulzer, Germany), with lesion windows parallel. Approximately 200 μm thick sections perpendicular to the lesion surfaces through the midline of both half lesions using an internal annulus saw microtome (Leica 160, Leica, Germany). The sections were then lapped down to 85 ± 5 μm using a RotoPol-21/RotoForce4 lapping instrument (Struers, Denmark) using 1200 grit lapping paper. Microradiography and computer-assisted microdensitometry were conducted on each section as described in detail previously.[12]

Each section contained two remineralized lesions and their paired demineralized control lesions. Lesion depth (Ld) of each scanned lesion was measured and densitometric profiles (vol%min versus μm) determined for each demineralized and remineralized lesion as well as for the median sound enamel. The difference between the area under the densitometric profile of the demineralized lesion and the median sound enamel, calculated by trapezoidal integration, is represented by ΔZd. The difference between the area under the densitometric profile of the remineralized lesion and the medium sound enamel is represented by ΔZr. These parameters were then converted to percentage change values after remineralization with respect to each control untreated demineralized lesion. As such percentage remineralization (%R) represents the change in ΔZ values:

$$\%R = \frac{\Delta Zd - \Delta Zr}{\Delta Zd} \times 100$$

2.6. *Saliva flow rate and ion analyses*

Subjects were instructed to swallow all saliva present in the mouth before collection started. Unstimulated saliva was collected by leaning forward with head tilted downwards and allowing saliva to flow into a pre-weighed 15 ml centrifuge tube for 2 min. Saliva stimulated by chewing sugar-free gum was collected using the same procedure.

Unstimulated and stimulated saliva samples were centrifuged (1000 × *g*, 15 min) and supernatants diluted, acidified

with 0.01N HNO_3, thoroughly mixed and transferred into a 10 ml syringe fitted with a 0.2 μm filter (Minisart, Sartorius, VIC, Australia). Filtrates were injected into a labeled Dionex sampling vial (Dionex Corporation, CA, USA). Levels of inorganic ions were determined using an automatic ion chromatography system, equipped with two columns for both cation (IonPac CS12) and anion (IonPac AS18) and two separated conductivity detectors (ICS3000, Dionex Corporation, CA, USA). A combined seven anion standard (#56933) and a combined six cation standard (#046070) were diluted 20, 50 and 100 times with distilled/deionised water to calibrate and quantify the conductivity reading.

2.7. Gum analyses

One dose (2 pellets or 1 slab) of each gum type was thinly sliced with a scalpel and extracted with either 40 ml of distilled deionised water or 40 ml of 0.01N HNO_3 overnight. Extracted samples were centrifuged (1000 × *g*, 15 min) to remove gum debris. Supernatants were collected, diluted and analyzed for pH (water extract only) and for ions using the Dionex system as described above. An extra series of sodium carbonate (Ajax Finechem, NSW, Australia) and tri-sodium citrate (BDH chemicals, VIC, Australia) standards were also included for carbonate and citrate ion analyses.

2.8. Data analysis

Data were statistically analyzed using repeated measures analysis of variance with contrasts.[17] Homogeneity of variance was tested using Levene's test and normality of the data was tested using normal probability plots and the Kolmogorov–Smirnov test. All statistical analyses were performed using SPSS version 12.0 software.[18]

3. Results

3.1. Gum ion analysis

Ion analyses of water and acid extracts of the four gums used in the clinical study confirmed that Trident Xtra Care and Orbit Professional contained added calcium (Table 2). Orbit and Extra are normal sugar-free gums without added calcium and the small amounts of soluble calcium were from the gum base. The highest amount of added calcium was found in Orbit Professional with 320 μmol of calcium in two pellets (single dose/serve) added as calcium carbonate with a small amount of calcium phosphate (Tables 1 and 2). However, most of this calcium was not extractable in water and required acid for solubilization. Only 69.70 ± 1.51 μmol of calcium ion was extracted into water from two pellets (3.13 g) of Orbit Professional, therefore the majority of the calcium added was unavailable. The analysis of this gum also confirmed the addition of citric acid/citrate which would account for some (69.70 ± 1.51 μmol, 22%) of the calcium phosphate/calcium carbonate being soluble (extracted) in water. The gum with the highest level of water-extractable calcium was the Trident Xtra Care with added calcium as CPP–ACP. This gum released 88.73 ± 2.59 μmol of calcium from one slab (1.96 g) into water.

3.2. Saliva ion analysis

Saliva samples were collected from the subjects while chewing the four sugar-free gums for the first 2 min of chewing. These saliva samples were analyzed for calcium and phosphate ions using the Dionex ion analyzer and the results are presented in Table 3. Chewing Orbit and Extra did not significantly increase saliva calcium and phosphate ion concentrations. However, chewing Trident Xtra Care and Orbit Professional did significantly increase salivary calcium concentration to 3.63 ± 1.73 and 3.95 ± 1.08 μmol/ml, respectively, and there was no significant difference between the gums in this respect.

The unstimulated and stimulated saliva flow rates for each of the 10 subjects ranged from 0.42 to 1.14 ml/min and 2.31 to 5.77 ml/min, respectively. The mean stimulated flow rate (4.22 ± 0.88) was approximately fourfold higher than the mean unstimulated flow rate (0.87 ± 0.21 ml/min) (Table 4). The mean stimulated saliva flow rates for the four sugar-free gums are presented in Table 4 and were not significantly different.

3.3. Enamel remineralization

All randomized subjects completed the *in situ* study without significant protocol violations and were included in the analysis. No adverse events were reported and no subjects withdrew. There was no correlation between stimulated saliva flow rates or saliva calcium levels and measurements of enamel remineralization ($\Delta Zd - \Delta Zr$ or $\%R$) (Table 4). Chewing Trident Xtra Care resulted in significantly ($p < 0.001$) greater

Table 2 – Ion analysis of water extracts of the four sugar-free gums.

Ion	Trident Xtra Care, μmol/dose	Orbit Professional, μmol/dose	Orbit, μmol/dose	Extra, μmol/dose
Ca^{2+}	88.7 ± 2.6[a,c,d]	69.7 ± 1.5[c] (320[b])	8.8 ± 0.3[c] (211[b])	24.0 ± 1.2[c] (266[b])
PO_4^{3-}	37.5 ± 0.9	0.38 ± 0.01	ND	ND
Citrate	ND	24.0 ± 0.1	ND	ND
CO_3^{2-}	15.0 ± 1.2	28.7 ± 5.1 (104[e])	9.0 ± 0.5 (82[e])	11.5 ± 0.1 (78[e])

[a] Mean ± SD.
[b] Total calcium, μmol/dose measured using 0.01N HNO_3 extraction.
[c] Significantly different ($p < 0.01$) to all other Ca values in row.
[d] Calcium added as CPP–ACP.
[e] Total carbonate, μmol/dose measured using 0.01N HNO_3 extraction; ND = not detected.

Table 3 – Calcium and phosphate ion analyses of saliva stimulated by chewing sugar-free gum.

Ion	Unstimulated saliva, μmol/ml	Trident Xtra Care saliva, μmol/ml	Orbit Professional saliva, μmol/ml	Orbit saliva, μmol/ml	Extra saliva, μmol/ml
Ca^{2+}	1.83 ± 0.47[a]	3.62 ± 1.73[b]	3.95 ± 1.08[b]	1.69 ± 0.26	1.99 ± 0.39
PO_4^{3-}	5.11 ± 1.68	4.83 ± 2.19	2.82 ± 0.75	3.49 ± 1.29	3.46 ± 1.49

[a] Mean ± SD.
[b] Calcium concentration in saliva significantly higher ($p < 0.01$) for Trident Xtra Care and Orbit Professional.

Table 4 – Saliva flow rates and enamel subsurface lesion remineralization by four sugar-free gums.

Gum	Gum stimulated salivary flow rate[a], ml/min	ΔZd, vol%min μm	Ld[d], μm	$\Delta Zd - \Delta Zr$, vol%min μm	%R
Trident Xtra Care	3.85 ± 0.65[b]	2006.89 ± 561.50	99.47 ± 9.08	414.87 ± 122.36[c]	20.67 ± 1.05[c]
Orbit Professional (with calcium)	4.72 ± 0.74	1792.73 ± 496.09	95.54 ± 8.50	222.86 ± 61.26	12.43 ± 0.64
Orbit	3.83 ± 0.92	2039.15 ± 305.94	96.55 ± 5.53	189.13 ± 27.10	9.27 ± 0.59
Extra	4.48 ± 0.91	2013.41 ± 418.32	97.63 ± 5.74	187.59 ± 34.83	9.32 ± 0.35

[a] Unstimulated saliva flow rate was 0.87 ± 0.21 ml/min.
[b] Mean ± SD.
[c] Significantly higher ($p < 0.01$) than other values in same column.
[d] Initial depth of the subsurface lesion.

enamel remineralization than that produced by chewing the other three gums (Table 4).

4. Discussion

This study utilized an intraoral enamel remineralization model to assess the remineralization efficacy of four sugar-free chewing gums, two with added calcium. The sugar-free gum Trident Xtra Care with added calcium as CPP–ACP was superior to the other three gums producing approximately double the level of remineralization. The gum Orbit Professional containing high levels (320 μmol calcium per two pellets) of calcium phosphate/calcium carbonate with citric acid released similar amounts of calcium to the Trident Xtra Care gum, however this did not result in increased enamel remineralization. Citrate anions bind calcium ions with the fully dissociated citrate anion exhibiting the strongest calcium binding [log *K* (CaH_2 Cit^+), 1.04; log *K* (CaH Cit^0), 2.03 and log *K* ($CaCit^-$), 3.64].[19] The binding of calcium ions by citrate will lower the calcium ion activity and therefore its remineralization potential. This suggests that even though the Orbit Professional gum released calcium, the ion was not bioavailable and hence did not promote enamel remineralization.

Calcium in the form of CPP–ACP has been shown to be bioavailable.[10,11,16] CPP containing the sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- have been shown to stabilize calcium, phosphate and hydroxide ions and to localize those ions onto the enamel surface.[10,11] The high solubility of the CPP–ACP in the Trident Xtra Care gum is shown in this study with the Trident Xtra Care gum releasing 88.73 μmol calcium/dose into water whereas the Orbit Professional gum, even with the very high level of calcium carbonate/calcium phosphate (320 μmol calcium/dose) and citric acid/citrate, only released 69.70 μmol of calcium/dose into water *in vitro* (Table 2). It should be noted that the dose (amount chewed per serve) of the Orbit Professional gum was significantly greater than the Trident gum dose hence the solubility differential is even greater when expressed per gram of gum. For example, the Trident Xtra Care gum released 45.3 μmol of Ca/g of gum whereas the Orbit Professional gum released only 22.3 μmol of Ca/g of gum into water *in vitro*. Interestingly, it appeared that the saliva calcium concentrations achieved on chewing the Trident Xtra Care (3.62 ± 1.73 μmol Ca/ml) and Orbit Professional (3.95 ± 1.08 μmol Ca/ml) gums were similar (Table 3) even though the Trident Xtra Care gum released more calcium into water *in vitro*. However, it should be noted that the saliva was collected while chewing the gum *in vivo* and it has been shown previously that CPP–ACP released by gum chewing binds to tooth surfaces.[10,16] Hence, it should be expected that the level of calcium from the CPP–ACP in saliva would be an underestimate of the CPP–ACP released due to the significant binding of the CPP–ACP onto tooth surfaces. These results highlight the important role of the CPP not only in markedly increasing the solubility of calcium and phosphate ions but also in binding and localising the ions at the tooth surface to facilitate subsurface enamel lesion remineralization.

The CPP–ACP nanocomplexes bind on contact with the enamel surface releasing the associated calcium, phosphate and hydroxide ions that are then available to diffuse down concentration gradients into the subsurface enamel to promote remineralization.[10,20] The bioavailability of calcium and phosphate ions of the CPP–ACP nanocomplexes and the promotion of subsurface enamel remineralization by the Trident Xtra Care gum in this *in situ* study are consistent with the recent caries clinical trial of Morgan et al.[13] This clinical trial demonstrated that chewing sugar-free gum containing CPP–ACP (Recaldent) relative to a control sugar-free gum over a period of 24 months resulted in a significant reduction in progression and enhancement of regression of approximal dental caries in school children. The control sugar-free gum was equivalent to the normal sugar-free gums used in the current study.

5. Conclusions

In conclusion, this *in situ* study demonstrates that Trident Xtra Care containing CPP–ACP was superior in remineralization of enamel subsurface lesions when compared with Orbit Professional, Orbit and Extra sugar-free gums. The study highlights the importance of calcium ion bioavailability in the remineralization of enamel subsurface lesions.

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