# Remineralization of enamel subsurface lesions *in situ* by the use of three commercially available sugar-free gums

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**Background.** Commercially available sugar-free chewing gums have been claimed to provide oral health benefits.

**Aim.** The aim of this randomized, double-blind crossover *in situ* study was to compare the efficacy of three commercially available sugar-free chewing gums: Trident White, Orbit, and Orbit Professional, in remineralizing enamel subsurface lesions *in situ*. **Design.** Specimens containing enamel subsurface lesions were sectioned into test and control half-slabs with the test half-slabs inserted into removable palatal appliances. For each test chewing period,

#### Introduction

The beneficial dental effects of chewing sugar-free gum, particularly decreased caries experience, have been reported<sup>1–3</sup>. The reduction in caries experience is most likely caused by increased salivary flow rates and increased levels of calcium and phosphate in gum-stimulated saliva limiting demineralization and enhancing remineralization<sup>4</sup>. However, claims for additional efficacy because of additives have been made. Present in some commercially available gums, the sugar alcohol xylitol has been claimed to have an effect in reducing dental caries<sup>5</sup>. Putative mechanisms of action include enhanced remineralization of enamel by

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subjects were randomly allocated one of three test gums. Subjects (n = 10) chewed the randomly allocated gum for a 20-min period four times per day for 14 days. Each subject chewed all three test gums, with a 7-day washout period between crossovers. After each 14-day cycle, test and control half-slabs were paired, embedded in resin, sectioned, and subjected to microradiography to determine remineralization. **Results.** The gum TW produced significantly greater remineralization ( $18.4 \pm 0.9\%$ ) than Orbit ( $8.9 \pm 0.5\%$ ) and Orbit Professional ( $10.5 \pm 0.9\%$ ). **Conclusion.** The superior remineralization activity of the TW gum *in situ* was attributed to the presence of casein phosphopeptide–amorphous calcium phosphate nanocomplexes.

xylitol acting as a carrier for calcium ions into white spot lesions<sup>6</sup>. A recent systematic review of clinical trials investigating the effects of xylitol challenged the greater caries reduction claimed for gums containing xylitol compared with other sugar alcohols<sup>7</sup>. Of the commercially available gums tested in this study, two gums [Orbit (Or) and Orbit Professional (OrP)] contained xylitol (Table 1).

The addition of casein phosphopeptideamorphous calcium phosphate nanocomplexes (CPP-ACP Recaldent, CASRN691364-49-5) to chewing gum has also been suggested to have an additive effect on the remineralization of enamel subsurface lesions (ESLs) to that of salivary stimulation<sup>8,9</sup>. It is claimed that the CPP-ACP nanocomplexes act as a calcium and phosphate reservoir incorporated into dental plaque and on the tooth surface, releasing calcium and phosphate ions to maintain a supersaturated environment with respect to tooth mineral, thereby reducing demineralization and enhancing remineralization<sup>10,11</sup>. The Trident White (TW) gum tested in this study contained CPP-ACP at 10 mg per serve (two pellets) (Table 1).

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Table 1. Manufacturer-listed ingredients of three commercially available chewing gums tested.

Orbit*	Trident White†	Orbit Professional*
Sorbitol	Sorbitol	Sorbitol
Mannitol	Maltitol	Xylitol
Xylitol	Mannitol	Mannitol
Acesulfame-K	Acacia	Aspartame
Aspartame	Aspartame	Acesulfame-K
Sucralose	Acesulfame-K	Gum base
Gum base	Gum base	Humectant
Glycerol	CPP-ACP‡	Glycerine
Lecithin	Sodium stearate	Gum arabic
Flavourings	Titanium dioxide	Flavourings
Butylated hydroxytoluene	Flavourings	Sodium polyphosphate
	Candelilla wax	Titanium dioxide (E171)
	Butylated hydroxytoluene	Brilliant blue (E133)
		Soy bean lecithin
		Carnauba wax
		Butylated hydroxyanisole

CPP-ACP, casein phosphopeptide-amorphous calcium phosphate.

\*Orbit and Orbit Professional (Wrigley Manufacturing Co., Chicago, IL, USA). †Trident White (Cadbury Adams USA LLC, Parsippany, NJ, USA).

‡CPP–ACP 10 mg per serve (two pellets).

The comparison of commercially available products provides information for the consumer (and prescriber) that directly relates to the purchased product, rather than relying on research that may relate to an active ingredient incorporated and tested in a different product and/or test environment.

The objective of this study was to compare the efficacy of three commercially available sugar-free chewing gums: Or (Wrigley Manufacturing Co., Chicago, IL, USA), TW (Cadbury Adams USA LLC, Parsippany, NJ, USA), and OrP (Wrigley Manufacturing Co.) in remineralizing ESLs in an *in situ* model.

The null hypothesis was that the remineralization outcome measure for the three chewing gums tested (Or, OrP, and TW) would not differ from each other.

# Materials and methods

### Ethics approval and study design

Ethical approval was obtained from the Human Research Ethics Committee of The University of Melbourne. All subjects provided informed consent. A randomized, double-blind crossover study with three different sugar-free chewing gums was conducted. The chewing gums were commercially available and were Or (2.0 g slab, Wrigley Manufacturing Co.) TW (1.5 g pellet, Cadbury Adams USA LLC), and OrP (1.5 g pellet, Wrigley Manufacturing Co.). The constituents of each gum as described by the respective manufacturers are presented in Table 1. The TW gum contained 10 mg CPP–ACP per two pellets (per serve). The gums were removed from their original wrappers, placed in coded wrappers, and then randomly assigned to the subjects. The gums could not be identified by the subjects by taste or appearance. The code for the three gums was not released to the investigators until the completion of the study and the statistical analysis of the data.

# Subject recruitment

Ten healthy adult subjects (six men, four women) were recruited from the staff and postgraduate students of the School of Dental Science, the University of Melbourne. Each subject had at least 22 natural teeth with no active caries, periodontal disease, or other oral pathology. None of the subjects was using antimicrobials or other medications that could affect saliva quality and/or flow. For each subject, the unstimulated salivary flow rate exceeded 0.2 mL/min, and the stimulated flow rate, measured after chewing two pellets of chewing gum exceeded 1.0 mL/min.

# Saliva collection

Unstimulated and stimulated saliva samples were collected at the start of the study. Unstimulated saliva was collected by subjects allowing saliva to flow for 5 min into test tubes by tilting their heads forward. Subjects were excluded if unstimulated saliva flow was less than 0.2 mL/min. Stimulated saliva was collected after subjects chewed two pieces of gum and collected stimulated saliva for 5 min into test tubes.

# Intra-oral appliances

Individual removable mid-palatal appliances retained by four stainless steel clasps were prepared as described previously<sup>11</sup>. These removable appliances held four test enamel half-slabs retained in bilateral troughs by sticky wax as described by Iijima *et al.*<sup>11</sup> to produce a 1 mm trough above the enamel surface to allow plaque to be established and retained.

# ESLs

Extracted human third molars were obtained from clinics of the Royal Dental Hospital of Melbourne, and stored in 10% (v/v) neutral buffered formalin. Sound, relatively planar buccal and lingual surfaces free of cracks, stains, and hypomineralized areas were selected and thrice rinsed with double-deionized water (DDW). The outer enamel surface was removed and polished wet using Soflex discs (3M ESPE, St Paul, MN, USA) on a slowspeed contra-angle dental handpiece. Each polished surface was then sawn from the tooth as a slab (approximately  $8 \times 4$  mm) using a water-cooled diamond blade saw (Minitom, Struers, Copenhagen, Denmark), and the slab was covered in acid-resistant nail varnish except for two (occlusal and gingival; approximately  $6 \times 1$  mm) windows. Subsurface demineralized lesions in the windows were prepared using a Carbopol C907 (BF Goodrich, Cleveland, OH, USA), calcium phosphate, lactic acid, pH 4.8 solution<sup>12</sup> for 96 h to create

subsurface lesions of 100  $\mu$ m in depth and with a surface layer of approximately 15  $\mu$ m. Following lesion preparation and varnish removal, the specimens were sawn into test and control half-slabs (Minitom, Struers), the four test halfslabs were inserted randomly into removable palatal appliances, and the control half-slabs were stored in a moist environment.

# Chewing regimen

The three chewing gums were allocated randomly to each subject for each of three 14-day treatment periods. Subjects chewed a recommended serve of each gum (TW, two pellets; OrP, two pellets; and Or, one slab) for a 20-min period four times per day (1000 h, 1130 h, 1400 h, and 1530 h). The subjects were instructed to remove their appliances when they ate, drank, or performed oral hygiene procedures. The appliances, however, could not be removed for at least 20 min after gum chewing had ceased. On removing the appliance, each subject was instructed to rinse it briefly with DDW and then store it in a sealed, humidified container (zip-lock bag with a small amount of deionized water) until re-insertion. After eating or drinking, the subjects were instructed to rinse their mouth with water and wait 20 min before re-inserting the appliance. Gum chewing was repeated daily for 14 days, followed by a 7-day washout period and continued until the subjects had chewed all three gums.

The subjects kept a compliance diary recording the times of chewing gum and appliance wear; the diary was collected at the end of each period. The subjects maintained normal oral hygiene and dietary habits during the study. Fluoride toothpaste (1000 p.p.m. F) was provided 1 week before the commencement of the study to be used for personal oral toothbrushing in the morning and evening throughout the study. The subjects were instructed to clean the appliances, avoiding the enamel slabs, with a toothbrush and fluoridefree toothpaste provided for this purpose. Following each treatment period, the enamel slabs were removed from the appliances, rinsed with DDW, and stored moist in labelled microcentrifuge tubes at room temperature.

Test and control half-slabs were paired with the lesion windows parallel and embedded in cold-cure methacrylate resin (Paladur, Heraeus Kulzer GmbH, Wehrheim, Germany). The resin vial was marked to identify test and control blocks, and the resin was allowed to set at room temperature overnight. Sections approximately 200 µm thick were cut from the embedded blocks perpendicular to the lesion surface through the midline of the lesions in the blocks using an internal annulus saw microtome (Leica 1600, Leica Microsystems GmbH, Nussloch, Germany). The sections were lapped down to  $85 \pm 5 \,\mu\text{m}$  using a RotoPol-21/ RotoForce4 lapping instrument (Struers) with 1200 and 2400 grit lapping paper, removed from the lapping instrument with absolute ethanol and rinsed in DDW, blotted dry, and stored on soft tissue between glass slides.

Each section (containing the test lesion and the control lesion from the same enamel slab) was microradiographed along with an aluminium stepwedge of  $10 \,\mu\text{m} \times 14 \,\mu\text{m}$  thick increments using Microchrome high-resolution glass plates (Type 1 A, Microchrome Technology, San Jose, CA, USA) and nickel-filtered copper K radiation at 20 kV, 10 mA for 6 min. Each glass plate was developed in 20 mL of Microchrome Developer D5 (1: 4 dilution, Microchrome Technology) for 4 min, placed into glacial acetic acid stop bath for 30 s, and then fixed in Microchrome Fixer F4 (1: 4 dilution, Microchrome Technology) for 4 min. The temperature of all the photochemicals was maintained at 20 °C by a water bath.

# Microdensitometry

Radiographic images of the lesions were viewed via transmitted light through a Dialux 20 microscope (Ernst Leitz, Welzlar, Germany), acquired by digital photography (Spot Insight Diagnostic Instruments, Inc., Sterling Heights, MI, USA) and analysed using imaging software Optimate version 5.2 (Orbotech, Yavne, Israel). Images of the lesions and neighbouring areas of sound enamel were scanned using the program's line luminance function giving readings in grey values between 0 and 256. An area

free of artefacts and cracks was selected for analysis. Each scan comprised 200 readings taken from the tooth surface across the lesion depth to the underlying sound enamel. The aluminium stepwedge image on each slide was scanned, and the average step grev value readings were plotted against aluminium thickness. The readings of the tooth section image lay within the linear portion of the stepwedge curve, and linear regression was used to convert grey values into equivalent thicknesses of aluminium. The section thickness was measured and the volume percentage (vol%) mineral data computed using the equation of Angmar *et al.*<sup>13</sup> and the linear absorption coefficients of aluminium, organic matter plus water, and apatitic mineral (131.5, 11.3, and 260.5, respectively). The image of the median strip between the two lesions was scanned six times and averaged to give a control sound enamel densitometric profile. The lesion images (test window and control window) on the gingival and occlusal sides of the median strip were scanned similarly, as close as possible to the median strip but avoiding any irregularities commonly found at the lesion edges, and the vol% mineral profiles were computed.

The differences in the areas (vol% min. µm) under the sound enamel profile, and the test and control profiles ( $\Delta Z$  values) were calculated from the densitometric profile of each lesion<sup>13</sup>. The percentage remineralization (%R) was calculated according to the formula,  $%R = (\Delta Z_d - \Delta Z_r)/\Delta Z_d \times 100$ , where  $\Delta Z_d$  represents the difference in the area under the sound enamel profile and the demineralized enamel profile, and  $\Delta Z_r$  represents the difference in the area under the sound enamel profile and the remineralized enamel profile<sup>14</sup>. The lesion depth (um) of each scanned lesion was recorded.

# Data analysis

Mineralization data of the ESL and stimulated salivary flow rates were analysed statistically by one-way analysis of variance and Scheffe's *post hoc* multiple comparisons test using a statistical analysis program SPSS, version 13.0 (SPSS, Inc., Chicago, IL, USA). The critical level for alpha was set at 0.05.

Sugar-free chewing gum	Gum-stimulated salivary flow rate (mL/min)	ΔZ <sub>d</sub> t vol% min. μm	ΔZ <sub>d</sub> – ΔZ <sub>r</sub> ‡ vol% min. μm	%R§	Initial lesion depth (μm)	
Orbit*	4.01 ± 1.25	2176.6 ± 127.3	191.2 ± 15.5	8.9 ± 0.5	95.1 ± 14.5	
Trident White*	4.23 ± 0.77	2642.1 ± 240.9	483.8 ± 53.1¶	18.4 ± 0.9¶	100.1 ± 19.5	
Orbit Professional*	4.47 ± 1.38	2425.4 ± 266.0	251.6 ± 48.4	$10.5 \pm 0.9$	98.2 ± 16.3	

Table 2.	Enamel	subsurface	lesion	parameters	and	stimulated	salivary	flow ra	ites.

\*(Mean  $\pm$  standard deviation) N = 10.

 $\pm \Delta Z_d$  = the difference between the area under the sound enamel profile and the demineralized enamel profile.  $\pm \Delta Z_r$  = the difference between the area under the sound enamel profile and the remineralized enamel profile.

\$%R = percent remineralization =  $(1 - \Delta Z_r / \Delta Z_d) \times 100$ .

¶Significantly different from other values in column (P < 0.001).



Orbit Trident White

**Fig. 1.** Representative microradiographic images of enamel subsurface lesions before (control) and after *in situ* remineralization by use of three commercially available sugar-free gums (Trident White, Orbit, and Orbit Professional).

### Results

#### Salivary flow rates

Unstimulated salivary flow rates (mL/min) at the start of the study ranged from 0.5 to 2.8, and stimulated flow rates during chewing Or, TW, and OrP were 2.1–6.3, 3.2–5.2, and 2.7–6.3, respectively. Mean flow rates (mL/min) after chewing Or, TW, and OrP were  $4.01 \pm 1.25$ ,  $4.23 \pm 0.77$ , and  $4.47 \pm 1.38$ , respectively

(Table 2). The mean stimulated flow rate  $(4.1 \pm 1.5 \text{ mL/min})$  was approximately three-fold higher than the mean unstimulated flow rate  $(1.3 \pm 0.8 \text{ mL/min})$ . There were no significant differences among the stimulated flow rates for the three gums tested.

# Remineralization values

There was no correlation between either unstimulated or stimulated salivary flow rates,

and percent remineralization observed with any of the three gums. The differences between vol% min. um values for the control and test mineralization profiles  $(\Delta Z_d - \Delta Z_r)$  were: TW, 483.8 ± 53.1; Or, 191.2 ± 16.0; and OrP,  $251.6 \pm 48.4$  (Table 2). The mean percent remineralization (%R) values were: Or,  $8.9 \pm$ 0.9% (range 7.9–9.7%); TW, 18.4 ± 0.5% (range 17.1-19.9%); and OrP,  $10.5 \pm 0.9\%$ (range 8.7-11.4%). The TW remineralization values  $(\Delta Z_d - \Delta Z_r \text{ and } \% R)$  were significantly higher than the corresponding values for the Or and OrP gums (Table 2). The difference in remineralization between the Or and OrP gums was not significantly different (Table 2). Representative microradiographic images of the enamel subsurface lesions remineralized by use of the three commercially-available gums and a control lesion are shown in Fig. 1.

# Discussion

In this study, significant differences were found between the chewing gums in remineralizing ESLs in an in situ model. This model controls for individual dietary and oral hygiene procedural differences, as the appliances were removed for all eating, drinking, and oral hygiene procedures, and therefore, the differences observed can be attributed to gum chewing alone. The TW gum replaced 107% more minerals than the Or gum and 75% more than the OrP gum. Saliva stimulated by chewing gum contains increased concentrations of calcium and phosphate<sup>4,15</sup> which can enhance remineralization of enamel<sup>16</sup>. Several constituents including carbamide, fluoride, xylitol, and sorbitol have been added to chewing gums to enhance the remineralizing effects of stimulated saliva. However, results have varied<sup>1-3,17-19</sup>, and any greater effectiveness above that of the chewing effect for these additives has been challenged<sup>3,7</sup>. This study compared the remineralization potential of three commercially available chewing gums with minor variations in basic constituents. Both xylitol and CPP-ACP have been claimed to increase enamel remineralization when added to sugarfree gum<sup>6,9</sup>. The results obtained with the xylitolcontaining gums (Or and OrP) in this study are similar to those obtained in previous studies of

the remineralizing potential of xylitol-containing sugar-free gums (pellets and slabs) using a similar *in situ* model<sup>9-11</sup>. In these previous studies, xylitol-containing sugar-free gums (Lotte Xylitol, Xylish, and Lotte Xylitol + 2 and other xylitol-containing gums) were shown to produce 6–9% remineralization when chewed for 20 min, four times per day for 14 days. These results are similar to those obtained with sorbitol-containing gums using a similar model suggesting that the remineralization efficacy is entirely because of the stimulated saliva as suggested previously<sup>9</sup>. The results obtained with the CPP-ACP gum are similar to those of 18-20% remineralization reported previously for CPP-ACP-containing sugar-free gum at a similar dose chewed four times per day for 14 days in a similar *in situ* model<sup>9-11</sup>. Hence, it is proposed that it is the presence of the CPP-ACP nanocomplexes in the TW gum that resulted in the TW gum being superior to the xylitol-containing gums (Or and OrP). The increase in remineralization was attributed to the ability of CPP-ACP to provide bioavailable calcium and phosphate ions at the enamel surface, to drive diffusion of the ions into the subsurface lesion to promote remineralization<sup>8–10</sup>. Previous studies of gums containing CPP-ACP have reported no detectable CPP-ACP in the gum bolus after 8 min of chewing<sup>9–11</sup>, illustrating effective release of the CPP-ACP from the gum.

In conclusion, the chewing of TW gum containing CPP–ACP produced 107% greater remineralization of subsurface enamel lesions than the Or gum, and 75% more remineralization than the OrP gum in an *in situ* model.

#### What this paper adds

- This paper adds knowledge about the differences in the *in situ* effect on remineralization of three commercially available chewing gums.
- The results support the evidence that CPP–ACP increases remineralization significantly when added to chewing gum by increasing availability of calcium and phosphate ions at the tooth surface.

#### Why this paper is important to paediatric dentists

- This paper provides information regarding the use of chewing gum which could be included in a preventive regime for children to decrease caries risk.
- The conclusions confirm the significant remineralizing effect of CPP–ACP, and reinforce the importance of calcium and phosphate ions in the remineralization of early caries.

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