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ORIGINAL ARTICLE

In situ randomised trial to investigate the occluding properties of two desensitising toothpastes on dentine after subsequent acid challenge

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Abstract
Objectives The aim of the study was to determine in situ the relative abilities of two desensitising toothpastes to occlude dentinal tubules with or without acid challenge.
Materials and methods The study design was a single centre, randomised, split mouth crossover model examining four treatments over two periods. The primary outcome was the degree of occlusion proffered by two desensitising toothpastes [Sensodyne® Rapid Relief (8% strontium acetate, 1040 ppm sodium fluoride) and Colgate® Sensitive Pro-Relief™ daily (8% arginine, 1450 ppm sodium monofluorophosphate)], a standard toothpaste (1450 ppm sodium fluoride) and water, after acid challenge. Healthy adult volunteers wore bi-lateral lower buccal appliances each with two dentine sections, receiving two treatments per study period. Samples were brushed twice a day with treatment, with two additional 3-min extra-oral acidic challenges applied ex vivo on days 3 and 4. A secondary outcome was the degree of occlusion attained in the absence of acid challenge. Examiners blinded to the study assessed occlusion by visual score of post-treatment scanning electron microscope images.
Results All 28 participants completed the study. In the absence of acid challenge, occlusion scores for both desensitising toothpastes were similar and significantly better than control scores ($p<0.02$). After acid challenge both desensitising toothpastes occluded more effectively than controls; however, occlusion scores for the strontium acetate paste were significantly greater than those of the arginine paste ($p<0.02$).
Conclusions The occluding properties of the strontium acetate toothpaste were significantly more robust after acid challenge than those of the arginine toothpaste.
Clinical relevance Patients with hypersensitivity, regularly imbibing dietary acidic drinks, should be advised that Sensodyne® Rapid Relief provides robust tubule occlusion despite repeated acidic challenges.

Keywords Dentine hypersensitivity · Dentine erosion · Toothpaste · Clinical study in situ · Dentine abrasion

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Introduction

Dentine hypersensitivity is a common and painful oral condition that, when professionally diagnosed, has been shown to affect approximately 15% of the population [1]. Dentine hypersensitivity, diagnosed when stimuli such as fluids or cold air elicit a short sharp pain, originates from exposed, patent, but otherwise clinically normal dentine [2]. Dentine can become exposed following gingival recession which has been associated with toothbrushing [3] or following other oral insults such as attrition or erosive attack that result in

loss of enamel or cementum [4]. It has been shown that a larger proportion of open tubules are present in hypersensitive teeth [5], and it is thought that for a patient to suffer hypersensitivity, dentine must be exposed and the dentinal tubules open from the oral environment to the pulp [6].

According to the hydrodynamic theory, the pain of dentine hypersensitivity results when a trigger contacts open dentinal tubules, causing a change in fluid movement within the tubule, which in turn stimulates pulpal nerve impulses [6, 7]. If fluid movement can be reduced by occluding open dentine tubules either fully or partially, hypersensitivity should also be reduced or eliminated. Evidence to support occlusion as a treatment modality to reduce the pain of hypersensitivity has been obtained from in vivo studies that have tested the efficacy of agents such as strontium acetate [8, 9], stannous fluoride/sodium hexametaphosphate [10] and arginine/calcium carbonate [11].

The mechanisms by which these toothpastes cause tubule occlusion vary; arginine/calcium carbonate pastes have been shown to plug dentinal tubules, and it has been suggested that this is achieved as a result of an agglomeration formed by the arginine and calcium carbonate which binds negatively charged dentine and within tubules [12]. Several recent studies have shown that a formulation containing 8% arginine in combination with calcium carbonate was more effective than a desensitising toothpaste containing 2% potassium ion, which reduces hypersensitivity by an alternative mechanism of interrupting the neural response to the stimulus [13–15].

Newer formulations of strontium based pastes have also recently been evaluated to determine their efficacy in relieving immediate and short term pain relief [16]. Strontium is thought to occlude dentinal tubules when it substitutes for calcium in hydroxyapatite and has also been shown to penetrate tubules [17, 18]. The abrasive filler, artificial silica, has also been shown to have a strong affinity for dentine [19], and in vitro and in vivo studies have suggested that it occludes tubules [20, 21]. Such formulations containing strontium acetate have also been shown in situ [22], and clinically, to be effective at reducing dentine hypersensitivity [8].

The relative abilities of strontium acetate and 8% arginine toothpastes to occlude dentinal tubules have recently been examined in in vitro studies that included an acid challenge to mimic the situation that occurs when a soft acidic drink is consumed. In these studies it was demonstrated that the strontium acetate paste occluded bovine dentine tubules more effectively than the arginine paste at all time points tested [23, 24]. However, an 8-week longitudinal in vivo study comparing 8% arginine/calcium carbonate and 8% strontium acetate pastes demonstrated that both desensitising pastes were very effective at reducing dentine hypersensitivity, with the strontium acetate paste being only significantly more effective than the arginine paste at 8 weeks when the challenge was tactile [25]. The in vivo study, however, did not include an acid challenge, and participants were asked to refrain from eating or drinking for 4 h prior to assessment. Hence, the effect of consuming an acidic challenge on the ability of these toothpastes to maintain reduced or eliminate pain from dentine hypersensitivity was not assessed. Therefore, further comparison of these products to determine their relative occlusion efficacies when subjected to acid challenge is warranted using in situ modelling utilising the benefits of the oral environment and mimicking the in vivo situation.

The aim of the present in situ study was to gain a clinical insight into the relative abilities of an 8% strontium acetate paste, an 8% arginine/calcium carbonate paste, a control fluoride paste and water to occlude dentinal tubules with and without a challenge from a common dietary acid following toothpaste treatment. The primary outcome was the effect after 4 days of treatment with an acid challenge on days 3 and 4, and secondary outcome after 2 days prior to acid exposure.

Materials and methods

Overview of study design

This in situ study was a single centre, single blind (blinded only to the persons responsible for performing the sample analysis), randomized, split mouth, four-treatment, two-period crossover design, in healthy subjects. The study evaluated the occluding efficacy of two desensitising toothpastes [Sensodyne (8% strontium acetate, 1040 ppm sodium fluoride) and Colgate (8% arginine, 1450 ppm sodium monofluorophosphate)], a non-occluding standard silica based toothpaste (1450 ppm sodium fluoride) and a Volvic® water control on human dentine with and without a subsequent dietary acidic challenge. Further details of the products tested are shown in Table 1. Occlusion was determined by four examiners blinded to the study, who were given representative scanning electron microscope (SEM) images (Phenom G2 pro desktop SEM, Lambdaphoto, UK) of the samples to score using a visual scoring index (1, occluded; 2, partially unoccluded; 3, equally occluded/unoccluded; 4, partially occluded; 5, unoccluded).

Randomised subjects wore bi-lateral lower buccal intra-oral appliances, each fitted with two dentine samples, with one product applied per appliance per period. Each of the subjects completed two treatment periods of 4 consecutive days; study periods were Monday to Thursday, or Tuesday to Friday. During treatment days the subjects wore their intra-oral appliances for a minimum of 5 h daily; appliances were removed from the mouth for a 1-h period over lunch. Treatment periods were separated by a washout period of at least 48 h, and at the beginning of each period two fresh

Table 1 Products used in this study

Retail name	Active ingredient(s)	Fluoride content
Colgate Sensitive Pro-Relief	Arginine/calcium carbonate (8 wt%)	Sodium monofluorophosphate (1450 ppm F^-)
Sensodyne Rapid Relief	Strontium acetate hemihydrate (8 wt%)	Sodium fluoride (1040 ppm F^-)
N/A: non occluding silica based benchmark paste	–	Sodium fluoride (1450 ppm F^-)

dentine samples were placed into each appliance for each subject. Subjects were given different products in each period, so that every subject received all four products during the course of the study. Toothpastes or Volvic® water were applied by the same operator extra-orally to the dentine samples twice a day, and then samples were rinsed with Volvic® water ensuring that all residue was rinsed from those treated with toothpaste.

On days 1 and 2 of each period, the appliances were worn for a minimum of an hour before the first and after the last treatment of the day. On days 3 and 4 of each period, study staff immersed the appliances in grapefruit juice extra-orally without agitation for 3 min and then rinsed them in Volvic® water, a minimum of an hour after each toothpaste treatment. After the second grapefruit juice challenge of the day, appliances were rinsed in Volvic® water but not returned to the mouth. At the start and the end of each day, appliances were disinfected in Corsydyl® mint mouthrinse for 3 min and then rinsed in water. For SEM imaging one sample from each appliance was removed after day 2 to assess the secondary outcome measure (no acid challenge) and the remaining sample after day 4 to assess the primary outcome measure (acid challenge included on days 3 and 4).

Subject recruitment and randomisation

The primary end point of this study was the mean occlusion score at day 4 (acid challenge included). Assuming the within subject standard deviation (Sw) at day 4 was 0.843 units as determined previously [26] and the use of a two-sided 5% significance test, 24 evaluable subjects were required to detect a difference of at least 0.5 units between the treatments with 80% power. Allowing for dropouts and protocol violations, sufficient subjects were screened in order to randomise 28 subjects, to ensure that 24 subjects completed the study.

The study was conducted in the Clinical Trials Unit at the Bristol Dental Hospital and School and ethical approval awarded by South West 2 Research Ethics Committee. Participants gave oral and written consent to take part in the study which was conducted to Good Clinical Practice guidelines. Eligible participants were all adults aged 18 or over in good general and oral health who were able to accommodate the lower bi-lateral intra-oral appliances. Exclusion criteria were pregnancy, lactation, current or recurrent disease or oral appliance/restoration(s) that could affect assessments, carious lesions, signs of dental erosion, susceptibility to acid regurgitation, oral soft tissue inflammation or disease, diabetes mellitus, xerostomia, medical disorder that may make the subject unlikely to complete the study, concomitant medication which has contraindications with grapefruit juice, known allergy to study materials, or participation in another clinical study within 30 days of the screening visit. The volunteers were enrolled from and randomised at Bristol Dental Hospital and School (the study site). At enrolment subjects were allocated a unique number assigned in ascending numerical order according to their appearance for screening. Subjects that met all the inclusion and exclusion criteria were randomised according to the randomisation schedule computer generated by the statistician without treatment decodes for the study site. This schedule randomised 28 subjects, with the additional four constituting a Latin Square balanced for carry over effect, and indicated for each subject and treatment period which product would be applied to which side of the mouth.

Preparation of dentine samples

Caries-free human third molar teeth that had recently been extracted from patients of 18 years or over and of either gender were used for the dentine samples. Teeth were obtained from the NHS Research Ethics Committee approved tooth tissue bank located at the study site which holds tooth tissue that has been collected with informed consent for studies such as these in accordance with the UK Human Tissue Act 2004. Following extraction, teeth were soaked in 20000 ppm available chlorine solution for at least 24 h, and then scraped clean of any remaining tissue with a scalpel. Teeth were sectioned at the cemento-enamel junction, and pulpal tissue was removed and then soaked for a further minimum of 24 h in 20000 ppm available chlorine solution. Roots allocated to the study were washed with copious amounts of water and then sectioned using a rotary diamond cutter (Microslice 2; Metals Research, Royston, UK) to produce dentine sections. Dentine sections were placed into polyurethane vacuum packed moulds and filled with the epoxy resin prior to polishing. Following embedding, the dentine surface to be exposed for treatment was

polished using 1200-grit carborundum paper to remove all epoxy resin. Samples were further polished with aluminium oxide powder (350 nm) slurried with water (1:10) to remove the bulk of the smear layer formed during the cutting process and ensure surface homogeneity. Each dentine sample was identified with a unique number on the reverse side of the dentine sample. Samples were etched using 10% citric acid for 30 s to achieve patent dentine tubules and then washed in copious amounts of distilled water. Each sample was examined by a non-destructive SEM (Phenom-World), and only samples with fully open dentinal tubules were admitted to the study. Baseline images of all samples were recorded.

Appliances were prepared by a designated orthodontic laboratory using standard techniques.

Details of study treatment

Enrolled subjects were given a standard toothbrush and toothpaste (Crest Decay Prevention Toothpaste, Procter and Gamble, Middlesex, UK; 1450 ppm sodium fluoride, 0.32% w/v) for their own twice daily oral hygiene (before 8:00 and after 21:00) for the duration of the study. Subjects were asked to abstain from using mouthrinses for the duration of the study, antacids, acidic medications or vitamin C preparations during study days and, during study hours, from smoking or chewing gum whilst their appliance was in situ. Drinks were limited on study days to tea, coffee and water, of which only water could be consumed without removing the appliance. There was no restriction on food types consumed for the duration of the study, but subjects were instructed to remove their appliance when eating and supplied with a moist pot in which to keep their appliance if it was out of their mouth for more than 2 min. On study days appliances were worn for a minimum of 5 h from 9:00± 30 min to 15:30±30 min with an hour when the appliance was removed at lunchtime.

Subjects attended the site twice a day at 10:00 and 14:30± 30 min for extra-oral product application by study staff. For toothpastes, 1.1 g was dispensed onto a pad and scooped onto a powered toothbrush, and then this was used to brush the samples in the appliance for 10 s, about 5 s per sample. After brushing samples were rinsed until toothpaste residue was no longer visible. Appliances treated with Volvic® water were immersed in this and brushed and rinsed as aforementioned. On days 3 and 4 subjects also attended the site at 11:30± 30 min for extra-oral administration of grapefruit juice by study staff. All the grapefruit juice was purchased from the same batch to standardise the acidic conditions, and appliances were immersed for 3 min after which they were rinsed with Volvic® water. A second grapefruit challenge and subsequent Volvic water rinse were undertaken at the end of days 3 and 4 at 15:30±30 min, but the appliances were not returned to the mouth after this second challenge. A single sample was removed from each appliance after day 2 and replaced with an acrylic blank and the remaining sample after day 4 for analysis by SEM.

The appliances were returned to the study site each day and stored overnight in a moist environment so that they did not dehydrate. Participants attended a follow-up visit within 7 days of the final treatment day to capture any adverse events.

Statistical methods

Four independent examiners blinded to the study scored SEM images captured at day 2 (in the absence of acid challenge) and day 4 (following acid challenge). The means of these scores were analysed using analysis of variance (ANOVA) performed to model the effects of subject, treatment, period and side of the mouth. As this was an exploratory study at both day 2 and day 4, pairwise comparisons of all test and control treatments were examined. To reduce the risk that significant differences were detected by chance, *F*-tests with 3 degrees of freedom from the ANOVA model were undertaken first before contrasts between pairs of treatments were interpreted. Our conclusions regarding efficacy of occlusion of the toothpastes tested are based only on differences where the corresponding *F*-test was statistically significant. ANOVA was used despite the ordinal nature of the data as 5-point ordinal scales as they tend to be close enough to Gaussian to ensure that the sampling distribution of the mean based on a reasonable sample size will be very close to Gaussian. The use of parametric tests is beneficial as it enables the reporting of interpretable point and interval estimates, and these analyses are more central to a proper interpretation than *p*-values. The distributional form of the data was checked by normal probability plots (*Q*–*Q*) plots for residuals from the four-way model by subject, period, side of the mouth and product. These plots indicated a close conformity of our data to Gaussian distributional form.

Treatment differences in mean occlusion score, corresponding 95% confidence interval of the treatment difference and the *p*-values of the treatment difference from this model are presented.

Results

Twenty-eight subjects were screened in late January 2011; all 28 were randomised and completed all study visits (31 January–17 February 2011). Of the subjects, 21 were female and 7 were male, with an average age of 42.4 years. Three subjects (10.7%) reported adverse events, but these were unrelated to the clinical trial and were classed as moderate in intensity. All day 4 specimens yielded usable data; however,

one day 2 specimen was identified as lost on removal from the appliance, and another was classed as unevaluable by all four scorers.

Because no participant dropped out of the study, it was not necessary to define distinct intention-to-treat (ITT) and per-protocol (PP) populations. All treatments were evaluated for statistically significant differences for the efficacy variables, mean occlusion score at day 2 in the absence of an acid challenge, mean occlusion at day 4 after acid challenge and change in occlusion score from day 2 to day 4. The main efficacy results are summarised in Fig. 1 and Tables 2–3, and examples of tubule occlusion at days 2 and 4 are shown in Fig. 2.

On day 2 (in the absence of acid challenge) the mean score was lowest (and therefore tubular occlusion the greatest) for Colgate Sensitive Pro-Relief daily followed by Sensodyne Rapid Relief, then water and then control paste (Fig. 1, Table 2). ANOVA (Table 3) indicated that the differences between the treatments were significant ($p<0.001$) and that both Sensodyne Rapid Relief and Colgate Sensitive Pro-Relief daily occluded tubules significantly better than the water and control paste ($p<0.02$). However, the difference in occlusion between Sensodyne Rapid Relief and Colgate Sensitive Pro-Relief daily was not significant at this time point in the absence of an acid challenge ($p=0.721$).

At the end of day 4, following repeated acid challenge and the application of the relevant brushed agent, the mean score was the lowest (and therefore tubular occlusion the greatest) for Sensodyne Rapid Relief, followed by Colgate Sensitive Pro-Relief daily, then water and then control paste (Fig. 1, Table 2). ANOVA (Table 3) indicated that the differences observed between the treatments were highly significant ($p<0.001$) and that Sensodyne Rapid Relief occluded significantly more tubules than any other treatment ($p<0.02$). Colgate Sensitive Pro-Relief daily occluded

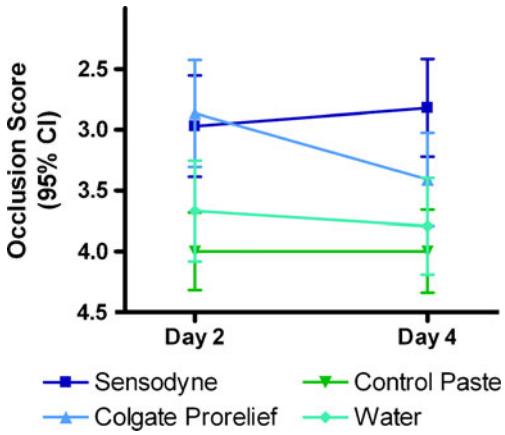

Fig. 1 Occlusion scores at days 2 and 4. Values are the means of the scores by four independent examiners with upper and lower 95% confidence intervals. The lower the score the greater the occlusion

Table 2 Summary statistics for occlusion score by scanning electron microscopy, by treatment

	Mean	Std. error	Std. dev	95% Confidence interval for mean	
				Lower bound	Upper bound
Mean score day 4					
Control paste	4.00	0.17	0.88	3.66	4.34
Colgate Pro-Relief	3.41	0.19	0.99	3.03	3.80
Sensodyne Rapid Relief	2.82	0.20	1.04	2.42	3.22
Water	3.79	0.19	1.02	3.40	4.19
Mean score day 2					
Control paste	4.00	0.16	0.81	3.68	4.32
Colgate Pro-Relief	2.87	0.21	1.14	2.43	3.31
Sensodyne Rapid Relief	2.97	0.20	1.06	2.55	3.39
Water	3.67	0.20	1.06	3.26	4.08
Change					
Control paste	0.00	0.26	1.35	−0.53	0.53
Colgate Pro-Relief	0.54	0.27	1.44	−0.01	1.10
Sensodyne Rapid Relief	−0.13	0.31	1.63	−0.78	0.52
Water	0.13	0.24	1.27	−0.37	0.62

Day 4 (primary), day 2 (secondary), and change from day 2 to day 4 (exploratory). Based on mean ratings by four observers

Table 3 Contrasts between pairs of treatments, at day 4 and day 2, and for changes in score from day 2 to day 4

Comparison	Contrast estimate	Standard error	95% Confidence limits		*p*-Value
			Lower	Upper	
Day 4					
Control vs. water	0.21	0.25	−0.28	0.69	0.41
Colgate vs. water	−0.38	0.25	−0.87	0.11	0.12
Sensodyne vs. water	−0.97	0.25	−1.46	−0.48	< 0.001
Colgate vs. control	−0.59	0.25	−1.08	−0.10	0.019
Sensodyne vs. control	−1.18	0.25	−1.67	−0.69	< 0.001
Colgate vs. Sensodyne	0.59	0.25	0.10	1.08	0.019
Day 2					
Control vs. water	0.28	0.28	−0.28	0.84	0.32
Colgate vs. water	−0.80	0.28	−1.35	−0.25	0.005
Sensodyne vs. water	−0.70	0.28	−1.26	−0.15	0.014
Colgate vs. control	−1.08	0.28	−1.64	−0.52	< 0.001
Sensodyne vs. control	−0.98	0.28	−1.55	−0.42	0.001
Colgate vs. Sensodyne	−0.10	0.28	−0.66	0.46	0.72
Change					
Control vs. water	−0.10	0.40	−0.89	0.69	0.80
Colgate vs. water	0.42	0.39	−0.36	1.20	0.29
Sensodyne vs. water	−0.30	0.40	−1.09	0.50	0.46
Colgate vs. control	0.52	0.40	−0.27	1.31	0.20
Sensodyne vs. control	−0.20	0.40	−0.10	0.61	0.63
Colgate vs. Sensodyne	0.71	0.40	−0.08	1.51	0.076

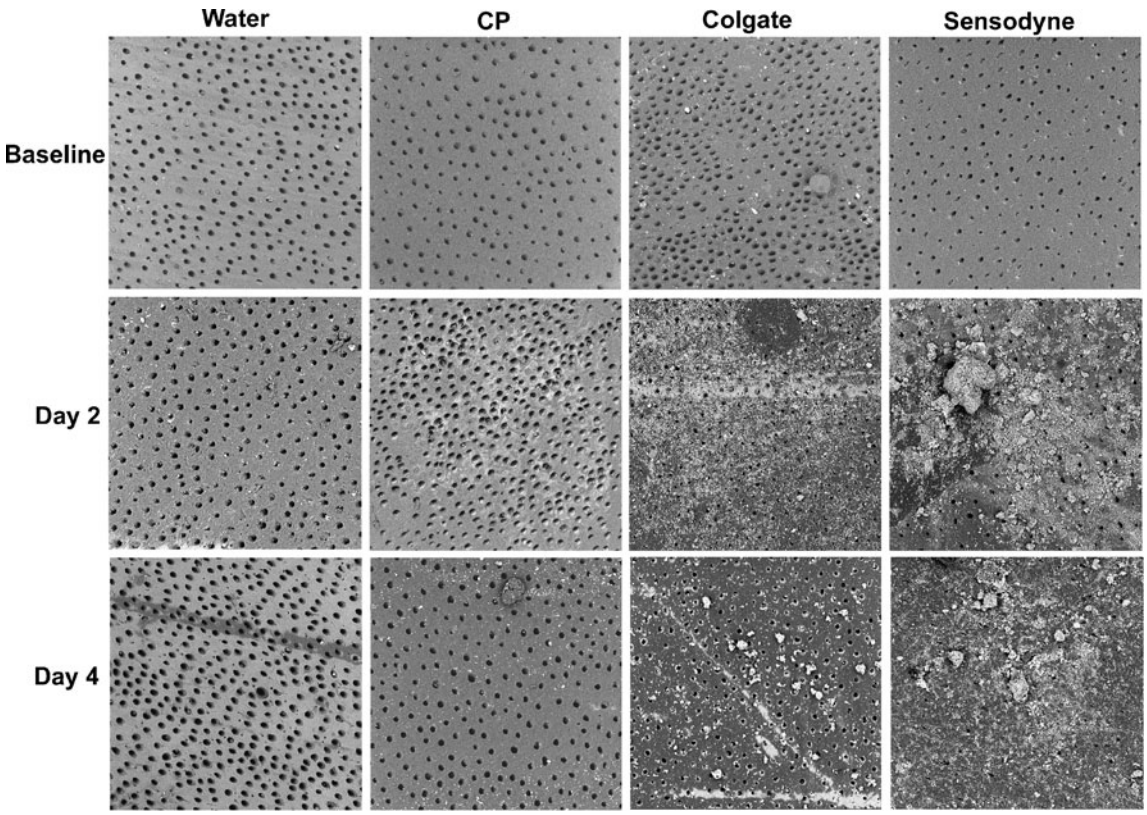

Fig. 2 Representative SEM images of dentine specimens showing the extent of tubule occlusion before treatment and following treatment with Colgate Sensitive Pro-Relief, Sensodyne Rapid Relief, a standard silica sodium fluoride toothpaste or water control after 2 days (no acid challenge) or 4 days (with grapefruit challenge)

significantly more tubules than the control paste ($p<0.02$), but the mean value was not significantly different from that of water ($p=0.122$). This comparison shows that Sensodyne Rapid Relief is superior to both water and the control paste in maintaining occlusion when subject to acid challenge, with Colgate Sensitive Pro-Relief daily intermediate in efficacy between Sensodyne Rapid Relief and the control paste.

There was also marginally statistically significant heterogeneity between subjects for the day 4 (following repeated acid challenge) results ($p=0.047$), but not for the day 2 (in the absence of acid challenge) results. This suggests that the volunteers do not appreciably contribute to biological variation.

Inter-examiner variation was detectable as a consequence of the very large number of specimens which were rated by all four examiners. However, when the variances between specimens and examiners were considered together with the error variance in the model, it was demonstrated that inter-examiner variation was not a major source of variation in the study.

Discussion

The identification of efficacious dentine occluding agents and the development of new product formulations to treat dentine hypersensitivity remains an important research focus as the condition affects an average of 15% of the population. Dental erosion has been suggested to be the most important factor in the initiation hypersensitive lesions of dentine [27] and is thought to be predominantly caused by extrinsic acids, such as those found in soft drinks, the consumption of which is increasing [28, 29]. The increase in the consumption of erosive drinks, coupled with increasing longevity of the healthy dentition [30] due to the emphasis on preventative dental treatments, suggests that the incidence of dentine hypersensitivity is increasing. As yet a single ingredient/product has not been identified as the treatment of choice for all cases [6].

This study examined the degree of occlusion provided by desensitising toothpastes (2), a control toothpaste containing silica and fluoride, or water in the absence or following an acid challenge. In the absence of acid challenge (day 2), it was demonstrated that both desensitising toothpastes occluded tubules to a similar degree ($p=0.721$) and provided significantly more tubule occlusion than the control paste or water ($p<0.02$). These findings are supported by a previous 8-week longitudinal study in vivo in which both toothpastes were shown to reduce dentine hypersensitivity to a similar degree (with the exception of tactile sensitivity at the final

time point [25]) and most likely reflect the fact that they have a similar mode of action. Both Sensodyne Rapid Relief and Colgate Sensitive Pro-Relief daily contain formulations that plug dentinal tubules through an interaction between their respective active ingredients, abrasive agents and the dentine itself [11, 21, 31]. Two previous in vitro studies demonstrated that in the absence of acid challenge, Sensodyne Rapid Relief provided significantly better tubule occlusion than Colgate Sensitive Pro-Relief daily using the same brushing strategy and amount of toothpaste [23, 24]. The reasons for the differences in findings between these in vitro studies and the present study are unknown, but may reflect the influences of study design with saliva flow, subject variation in saliva composition as well as the fact that samples were exposed to a normal oral environment in this in situ study and were of human rather than bovine origin.

In the current study, the addition of a dietary acidic challenge revealed differences between the efficacies of the desensitising pastes to occlude dentinal tubules, with, at day 4, Sensodyne Rapid Relief occluding significantly more tubules than the arginine/calcium carbonate based Colgate Sensitive Pro-Relief daily toothpaste ($p<0.02$). This data is supported by the two previous in vitro studies that have compared tubule occlusion of these toothpastes when followed by an acid challenge [23, 24]. In another in vitro study that examined tubule occlusion, it was shown that Colgate Sensitive Pro-Relief daily was not as resistant as other products to a citric acid challenge and, furthermore, that following citric acid treatment tubule occlusion was reduced from 91 to 54% [32]. The data obtained in the present study also showed that the occlusion efficacy of Colgate Sensitive Pro-Relief daily fell following acid challenge, while that of Sensodyne increased slightly. These findings collectively suggest that arginine based pastes are acid labile over time.

It might be anticipated that arginine based pastes would occlude dentinal tubules less well under acid conditions as arginine, positively charged at physiological pH, is thought to form a positively charged agglomeration with calcium carbonate and then bind to the negatively charged dentine [12]. Under acid conditions charge will be lost, and this may lead to the agglomeration dissociating from the dentine, although the toothpaste does contain bicarbonate to help buffer against pH changes [11]. A recent study by Petrou et al. [31], however, tested the resistance of arginine/calcium carbonate based toothpastes to acid challenge and demonstrated that after 2 min in cola (pH 3.2) dentine tubules remained occluded. The reason for the differences in findings between the study by Petrou et al. [31] and those of others is unclear, but likely to reflect differences in the protocols used. Prior to acid challenge Petrou et al. [31] treated samples repeatedly with toothpaste for a total of 5 min as compared to 10 s [23, 24], and the acid challenge used was 2 min in a cola (phosphoric and citric acids) with agitation as compared to 5 min in grapefruit juice or citric acid with no agitation [23, 24, 32]. In the current in situ study, toothpaste treatments were designed to reflect an in vivo brushing regimen, with a more aggressive acidic challenge of grapefruit juice, limited to 3 min without agitation. The erosive challenge was chosen to be at the harsh end of the spectrum, so that it could be determined whether occlusion was maintained in a harsh erosive regime. By monitoring the pH at the tooth surface, Millward et al. [33] showed in vivo that following the consumption of 1% citric acid drink, the pH remained below 5.5 for 2 min and 4–5 min at the palatal surfaces of an upper central incisor and an upper first molar, respectively, suggesting that this length of challenge occurs in vivo.

While in the present study there was some suggestion that the arginine based toothpaste Colgate Sensitive Pro-Relief daily was less effective when an acid challenge was introduced (by day 4), there was no evidence that the strontium toothpaste occluded less well under these circumstances. These findings are supported by previous in vitro studies [23, 24]. That strontium acetate pastes form occluding layers that are resistant to acid challenge has been demonstrated previously in an in situ study [21]. In this study occlusion efficacy of a strontium acetate paste containing artificial silica was determined with or without an orange juice challenge, and only modest differences were observed in tubule occlusion efficacy. It has previously been shown that artificial silica in the absence of an ionic detergent adsorbs strongly to dentine and is resistant to acid challenge [19]. The strontium acetate toothpaste tested in the present study contained artificial silica without an ionic detergent, and therefore the artificial silica and detergent may be responsible, at least in part, for its acid resistance.

As dental erosion has been shown to be a pre-requisite for dentine hypersensitivity [27], other strategies that inhibit erosion may help to reduce the incidence of dentine hypersensitivity. For example, Hughes et al. [34] demonstrated that the addition of a high concentration of calcium in the form of calcium carbonate to a blackcurrant fruit drink reduced erosion. Similarly, more recently it has been shown that modification of orange juice using an effervescent tablet containing calcium carbonate amongst other things reduced erosion to levels similar to a water control [35]. This data supports the theory that erosive potential depends on both the pH of a drink and the degree of saturation it has with respect to tooth minerals. Using a similar strategy, nanoparticles of hydroxyapatite were added to a sports drink and shown to inhibit erosion in a dose-dependent manner [36]. Products containing casein phosphopeptide amorphous calcium phosphate (CPP-ACP) in which the phosphopeptide stabilises the ACP such as Tooth Mousse® have also been

developed and shown to offer some protection against erosion involving toothbrush abrasion [37]. These strategies, however, are preventative and may not benefit those who already have dentine hypersensitivity.

The present study was undertaken in situ, but one compromise with this approach is that simulation of pulpal pressure is not possible in this type of study as a vital tooth cannot be used. Simulation of pulpal pressure can be achieved in vitro using Pashley models, but such studies lack the influence of the oral environment. In vivo studies to test the efficacy of desensitising toothpastes can and are undertaken, but it is difficult to determine/confirm the mechanisms of action of a desensitising toothpaste. Furthermore, although in vivo studies that recruit patients with dentine hypersensitivity will yield the best data regarding efficacies of treatments to reduce dentine hypersensitivity, in practice results obtained can be difficult to interpret. Patients often have different pain thresholds, and analysis of data collected can be complicated by the placebo effect which is well documented in dentine hypersensitivity studies [38, 39]. We specifically wanted to determine whether the toothpastes used in this study blocked the dentinal tubules and whether blocking was retained after acid challenge when tested in an oral environment, and the in situ model was selected as the most appropriate to test this in an environment as close to in vivo as possible. In in situ studies, such as the present study, an environment is provided close to that found in vivo, which is well controlled and provides a good model in which to evaluate desensitising dentifrices. Although better controlled than in vivo trials, in situ trials contain more variables than in vitro studies, and in the present study the residual variation in the analysis was greater than anticipated. Nevertheless clear differences between the treatments were demonstrable using this validated model to investigate tubule occlusion.

In conclusion, this study has shown that both Sensodyne Rapid Relief and Colgate Sensitive Pro-Relief daily offer good dentine tubule occlusion. However, in the presence of a dietary acidic challenge, the occlusion provided by Sensodyne Rapid Relief is clearly superior. This finding is of significance clinically as a causal relationship between erosion and dentine hypersensitivity has been demonstrated [27], and the consumption of soft drinks, a major risk factor for erosion, is increasing [28]. Furthermore, it has been shown that patients suffering from this condition consume more acidic beverages than non-sufferers [40]. Sensodyne Rapid Relief may, therefore, provide more sustained pain relief from dentine hypersensitivity in patients who are unable or unwilling to reduce their soft drink consumption as occlusion is less acid labile.

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Conflicts of interest None of the authors are aware of any conflict of interest arising from conducting and reporting the findings of this study.

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