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Clinical studies to determine the effectiveness of a whitening toothpaste at reducing stain (using a forced stain model)

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Abstract: *Aims:* Two single centre, randomized single-blind, crossover studies were performed, to compare the effect of a test toothpaste with a conventional fluoride paste in the inhibition and removal of extrinsic dental stain promoted by repeated chlorhexidine/tea rinses. *Methods:* These studies used 24 subjects in each of two separate clinical trials. On the Friday before each trial period, the subjects received a prophylaxis to remove all staining, plaque and calculus deposits. On the following Monday, subjects were checked whether they were stain free and then under direct supervision they rinsed with a 0.2% chlorhexidine mouthrinse, immediately followed by a rinse with a warm black tea solution. This cycle was repeated hourly eight times throughout the day and on the following days until the Friday. In addition subjects also received daily a single toothpaste slurry rinse or control water rinse in the morning and lunchtime. No other form of oral hygiene was permitted during this period. On the Friday, both stain area and intensity was assessed using the Lobene Stain Index. For the stain removal study, stain was promoted again using chlorhexidine and tea rinses. After 4 days, stain was measured both prior to and immediately after brushing with the allocated toothpaste for 2 min. Subjects were then instructed to use the toothbrush at home according to their normal oral hygiene practices. On the following Wednesday, the amount of stain present was re-assessed. Each subject subsequently received a thorough prophylaxis to remove all plaque calculus and staining before starting the following periods of the study. *Results:* The study showed no difference in the ability of the test whitening toothpaste, control toothpaste and water control at inhibiting stain. There was also only a small difference (3.5% for product of area

and intensity) between the ability of the two toothpastes to help remove stain after a single brushing. The difference was however in favour of the test product which approached a conventional level of significance ($P = 0.089$). There was no evidence of superiority for either of the pastes after normal home usage. *Conclusions:* This study has suggested that the test product may have some advantage over the conventional paste at removing stain but the magnitude of difference would appear to be small and of little clinical relevance.

Key words: dental stain; chlorhexidine; toothpaste; clinical trial

Introduction

Over the last few years there has been considerable interest in the use of whitening toothpastes to reduce remove extrinsic dental staining, with more and more products becoming commercially available. The mode of action of many of these products would appear to rely on the incorporation into the formulation of an effective abrasive system and/or chemicals that could help to inhibit or remove stain. The chemicals used centre around the use of surface active agents, whitening bleaches or oxidizing agents (1). The aetiology of extrinsic stain may have more than one origin, however dietary factors are implicated as a major source of stain. Similarly, the antiseptic chlorhexidine is also known to produce staining through an interaction on the tooth surface with dietary chromogens such as those found in tea, coffee and other beverages (2, 3). This latter phenomenon can be exploited in clinical studies to force stain production in subjects over relatively short periods of time. Thus in a matter of days large amounts of stain can be developed through multiple daily rinses with chlorhexidine and tea instead of home rinsing with twice daily rinses of chlorhexidine alone over periods of weeks (4). The efficacy of toothbrushes or whitening toothpastes can then be evaluated at removing this staining (4–6) when used in a normal home setting. Similarly, the ability of test toothpaste to inhibit stain deposition can also be assessed by the use of the toothpaste during the stain build up period. In the present study these models were used to evaluate the effectiveness of a novel whitening toothpaste at removing and inhibiting dental stain, after and over a 4-day period of stain accumulation. The test paste was based on a formulation which had shown in the laboratory to have very good physical stain control (pellicle cleaning ratio) with low abrasivity (relative dentine abrasivity) (data on file). The design of the study would be compatible with determining the combined physical and chemical effects on stain removal and on the chemical effects on stain inhibition alone. For comparative purposes, the effects of a conventional fluoride paste was also assessed and for the stain inhibition study a water rinse was also used as a control.

Materials and methods

A group of 24 healthy dentate volunteers were recruited for each study who fulfilled the necessary inclusion/exclusion criteria. Prior to the study, approval form the local Ethics Committee was sought and fully informed consent, both oral and written, was obtained from all participating subjects. The study toothpastes consisted of a test paste with a novel whitening formulation and a commercially available fluoride toothpaste (Boots Freshmint Fluoride: Boots Group PLC, Nottingham, UK) applied by using a commercially available toothbrush. The test paste contained an optimized physical cleaning system (high cleaning, low abrasive silicas) with a chemical stain controlling (whitening) agent, sodium tripolyphosphate. For a water control, a commercially available mineral water (Volvic: Danone Waters, London, UK) was used. The design of the studies used 4-day stain formation periods, two for the removal study and three for the inhibition study. Each subject was assigned to one of the toothpastes or control water rinse according to a predetermined randomization schedule supplied by the sponsor. Prior to each study period, each subject received a thorough prophylaxis to remove all staining, plaque and calculus from the dentition. On day 1 of the treatment phase (Monday), the teeth were examined to confirm that they were stain free. Any remaining stain required the subject to undergo a further oral prophylaxis. At this time and for the following 3 days, subjects were instructed under supervision to rinse with 10 ml of a 0.2% chlorhexidine mouthwash

for 60 s eight times a day and then expectorate. Immediately after rinsing with the mouthwash, the subjects rinsed for 60 s with 10 ml of a warm tea solution and then expectorated. For the inhibition study, additional rinses with the test/control toothpastes (3 g/10 ml) or water control were taken during the forced stain periods once in the morning and once at lunchtime. Throughout this period, volunteers omitted all other forms of oral hygiene except rinsing with the chlorhexidine mouthwash. This regimen continued each day until the Friday when the level of stain on teeth was assessed by an experienced clinician (NC), who had extensive experience in stain assessment and had been calibrated in the past from other studies (7, 8).

Using the method described by Lobene (9), the intensity of stain on the gingival crescent and body of the tooth on the buccal surfaces of each assessable incisor, canine and premolar and lingual surfaces of all incisors and canines were observationally scored using the four-point scale: 0, no stain; 1, light stain; 2, moderate stain; 3, heavy stain.

Using the method described by Lobene (9), the area of stain on the gingival crescent and body of the tooth on the buccal surfaces of each assessable incisor, canine and premolar and lingual surfaces of all incisors and canines were observationally scored using the four-point scale: 0, no stain detected only tooth colour; 1, stain covering up to one-third of the tooth surface; 2, stain covering between one-third and two-thirds of the tooth surface; 3, stain covering more than two-thirds of the tooth.

On the scoring day for the stain removal study (Friday), the previous index was used to assess subjects stain levels again by an experienced examiner (JM) who also had extensive experience in stain assessment and had been calibrated in the past from other studies (10, 11).

The subjects were then given their allocated test or control toothpaste and toothbrush to clean their teeth. The paste was applied by the clinical trial assistant to cover the entire bristle surface of the brush head. Subjects then brushed their teeth with the supplied toothpaste as they normally would for 2 min. Subjects immediately returned to the clinic where their teeth were re-scored for staining by the clinical assessor and re-photographed. They were then told to brush at home with the allocated toothbrush and provided toothpaste until the Wednesday of the following week. When returning to the clinic, subjects were re-scored for amount of stain and their teeth photographed. Each subject also received a thorough prophylaxis to remove all plaque calculus and staining before the second study period.

For the stain inhibition study, levels of stain on the dorsum of the tongue after 4 days stain formation were also assessed using the Lobene Index.

The following periods of the study employed the same regimen and on completion of each leg of the study, volunteers were seen again to remove any deposits of stain, plaque and calculus.

Statistical analyses

The primary outcome measures for both studies were the whole-mouth mean stain area score and stain intensity of the mean combined (product of area and intensity) dental stain scores on the assessment day. The primary outcome measures were summarized by calculating mean and standard deviation for each treatment. Mean values and standard deviations were also calculated where appropriate, for each tooth subsets of tooth surface, i.e. gingival crescent and body of tooth, lingual and buccal surfaces. For the inhibition study, the main analysis was ANOVA corresponding to the crossover design, modelling the stain score on three factors, subject, period and treatment. Point estimates, 95% confidence intervals and *P*-values were calculated for differences between the three treatments. Preliminary examination of data did not suggest any serious departure from Gaussian distributional form and as such confirmatory nonparametric analyses were not warranted.

For the stain removal study the Hills–Armitage method was used to analyse the data for each of the primary outcome variables of period and treatment. Point estimates, 95% confidence intervals and *P* values were calculated for differences between the two treatments. For possible non-Gaussian data distribution, confirmatory nonparametric Mann–Whitney tests were also performed.

Results

For the stain inhibition study, a total of 24 subjects comprising four males and 20 females (age range 20–54 years, mean = 34.6 years) was recruited. Data of all the subjects who completed the study were included. None of the subjects were either suspected or known to have seriously violated the protocol. Of the 24 subjects, 23 completed all test periods. For each study period, significant amounts of stain was evident irrespective of treatment with the toothpastes or water control (Table 1). Essentially there was little difference between the ability of the two toothpastes to inhibit stain compared with each other or water control (Table 2). This was evident when assessing stain area, intensity or a product of the two measurements. These findings were also consistent when considering separately gingival and body sites, lingual and buccal sites (results not presented here). Similar lack of significant

Table 1. **Stain inhibition after 4-day use of toothpaste slurries or water control**

	Average stain intensity (I)	Average stain area (A)	Average stain product (I × A)
Test paste	1.89 (0.42)	2.40 (0.37)	4.71 (1.30)
Control paste	1.90 (0.43)	2.39 (0.33)	4.69 (1.39)
Water	1.91 (0.55)	2.32 (0.37)	4.67 (1.76)

Summary statistics for stain intensity, area and product are based on all assessed sites.
Values are given as mean (SD).

Table 2. **Stain inhibition after 4-day use of toothpaste slurries or water control. Differences between test toothpaste, control paste and water control are based on all assessed sites**

	Point estimate	95% Confidence interval	*P*-value
Intensity (I)			
Test versus control	−0.03	−0.19 to +0.13	0.70
Test versus water	−0.04	−0.20 to +0.12	0.59
Area (A)			
Test versus control	+0.03	−0.12 to +0.17	0.71
Test versus water	+0.09	−0.05 to +0.24	0.20
Product (I × A)			
Test versus control	−0.02	−0.48 to +0.45	0.94
Test versus water	+0.01	−0.46 to +0.47	0.97

differences between treatments for assessment of tongue staining was noted (results not presented here). ANOVA for tooth and tongue staining demonstrated a period effect with less stain scored from period 1 to period 3. This did not influence the subsequent analyses and interpretation of results.

For the stain removal study, a total of 24 subjects comprising nine males and 15 females (age range 20–58 years, mean 33 years) was recruited. Data were included on all of the subjects who completed the study. None of the subjects were either suspected or known to have seriously violated the protocol. Of the 24 subjects, 21 completed all three test periods. The findings of the study essentially showed there was relatively little difference between the ability of the two toothpastes to remove stain at a single test brushing or after 5-day home usage when assessing stain area, intensity or a product of the two measurements (Table 3). As expected, brushing with either toothpaste was seen to remove significantly large amounts of stain following a single brushing and following a week's home usage. Stain left following use of the brushes was mostly found at the gingival margin. Analyses of a product of area and intensity showed differences between the two toothpastes after a single brushing were small (3.5%) but tended to favour the test toothpaste for all sites ($P = 0.089$) and buccal sites alone ($P = 0.085$) (Table 4). Following 5-day home usage

Table 3. **Stain removal following single brushing with toothpastes and 5-day home use of toothpastes**

	Average intensity (I)	Average area (A)	Product (I × A)
Test paste			
Pre-brushing	2.24 (0.40)	2.16 (0.60)	5.27 (1.55)
Post-brushing	0.94 (0.43)	0.47 (0.26)	1.14 (0.69)
% Remaining	41.91 (14.61)	23.11 (11.11)	22.37 (10.08)
5-day home use of paste	0.52 (0.37)	0.26 (0.22)	0.59 (0.53)
Control paste			
Pre-brushing	2.14 (0.35)	2.02 (0.52)	4.83 (1.40)
Post-brushing	0.93 (0.33)	0.49 (0.23)	1.19 (0.55)
% Remaining	43.70 (14.38)	25.59 (12.58)	25.91 (11.56)
5-day home use of paste	0.57 (0.34)	0.27 (0.18)	0.63 (0.44)

Summary statistics for stain intensity, area and product are based on all assessed sites.
Values are given as mean (SD).

Table 4. **Differences between control and test toothpastes in percentage of stain remaining after a single brushing**

	Point estimate	95% Confidence limits	*t*-ratio	*P*-value	*P*-value from Mann–Whitney test
All sites					
Intensity	−1.63	−9.24 to +5.99	−0.45	0.66	0.36
Area	−2.32	−6.36 to +1.72	−1.21	0.24	0.16
Product	−3.47	−7.53 to +0.59	−1.79	0.089	0.091
Buccal sites					
Intensity	−2.20	−0.39 to +5.99	−0.56	0.58	0.36
Area	−2.42	−5.75 to +0.91	−1.52	0.15	0.16
Product	−3.64	−7.83 to +0.56	−1.82	0.085	0.091

no significant differences were found between either toothpaste ($P = 0.49$ for mean stain product). No untoward side-effects were noted.

Discussion

At present there would appear to be considerable demand for oral hygiene products which whiten teeth by eliminating or reducing extrinsic dental stain. The incorporation of abrasives such as the high cleaning, low abrasive silicas used in the test paste may help to physically remove stain but as virtually all toothpastes contain abrasives some benefit may be expected even by conventional products. The concept of whitening formulations containing specific chemicals which reduce or inhibit stain independent of a physical effect would appear to be particularly attractive because reduced staining may be apparent in sites of the dentition where the abrasive effects of the toothpaste would be less obvious. To date various types of chemicals have been suggested to be of potential value and include surfactants, enzyme systems, calcium chelating builders and calcium phosphate absorbants (1). These chemicals work in a variety of ways but clinical evidence of efficacy to support laboratory data remains patchy (12). In the present study, a novel test formulation containing a chemical whitening agent, sodium tripolyphosphate was evaluated to determine whether stain could be inhibited more effectively than by a conventional paste. This chemical, sodium tripolyphosphate has been shown *in vitro* to inhibit initial stain formation, retard its further development and indeed remove it once formed (R.P. Shellis, unpublished data). As there were no significant differences between the test paste and the control, the chemicals in the test paste did not confer any additional benefit at inhibiting stain. Perhaps inactivation or reduced activity of active whitening chemicals, because of inappropriate formulation, may have accounted for the disappointing effects of the test toothpaste. Equally, neither of the pastes inhibited stain compared with the water control and as such no chemical inhibition of stain by either of the toothpastes could be demonstrated.

Similarly the novel test formulation was evaluated to determine whether preformed stain could be removed more effectively than by a conventional non-whitening toothpaste when applied by using a toothbrush. Whether used in a single brushing or following 5-day home usage, little benefit could be demonstrated for the test paste over the conventional paste. Using the toothpastes with a toothbrush could benefit stain removal by both a physical (abrasive) and chemical action. Both pastes applied this way were seen to be associated with a reduction in stain area and intensity. For stain area this could be expected simply through the abrasive effects of the pastes and the physical use of the toothbrush. The reduction in stain intensity is a bit harder to explain. It is possible however that abrasives and perhaps a combination of abrasives and chemicals alter the physical nature of established stain. Subsequently reduction in intensity may simply be the result of (i) thinning of stain layers by the abrasive action of the toothbrush and toothpaste, (ii) alteration of stain colour characteristics, e.g. brown to yellow by a chemical action. As there was little difference in staining following use of the two pastes it is likely that a common ingredient in both, such as detergent, could account for any chemical effect if present. Indeed, a detergent such as sodium lauryl sulphate could reduce stain, as has been shown in previous laboratory studies (13). Any whitening ingredients formulated in the test paste, such as sodium tripolyphosphate, may have had an additional benefit but as demonstrated in these studies, the effect would appear to be small and of little clinical benefit.

It is difficult to speculate as to why the PCR laboratory model demonstrated good physical stain control for the test paste while the clinical study showed little advantage for the product at reducing chlorhexidine-induced stain when compared with the control paste. One possibility is that the laboratory model is not a good predictor of efficacy at reducing chlorhexidine-induced stain in the mouth. This may be the result of the nature of chlorhexidine stain itself that may become calcified in the oral environment. Certainly there is evidence that acquired pellicle exposed to chlorhexidine calcifies to some extent (14) which is consistent with reports of an increase in supragingival calculus seen with the use of the antiseptic (15, 16).

In conclusion, the findings of the study would imply that the test paste may confer some additional stain removal properties beyond that of the conventional paste, but the benefit would be of little clinical advantage. On the basis of the present results, the stain inhibition and removal properties of the test toothpaste were re-evaluated in a laboratory model, using chlorhexidine/tea induced staining (17) and was shown to have little effect. As a result it was subsequently decided to change the experimental formulation, with the aim to achieve both better physical and chemical stain control.

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