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Development of methods to enhance extrinsic tooth discoloration for comparison of toothpastes

2. Two-product clinical study

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Abstract

Background: Extrinsic staining of teeth is considered to be unsightly and a number of 'whitening' toothpastes have been formulated to inhibit or remove such tooth discoloration. The aim of this study was to compare the stain prevention of two toothpastes.

Method: The study was a single-blind, randomised, placebo-controlled, crossover design, balanced for residual effects involving 24 healthy dentate volunteers. The treatments were as follows: (1) a whitening toothpaste product, (2) an experimental toothpaste formulation and (3) water. For each 4-day rinse period, subjects were rendered stain free on the teeth and tongue. Approximately on the hour from 09:00 to 16:00 hours, subjects rinsed with chlorhexidine mouth rinse for 1 min followed by warm black tea for 1 min. The treatment interventions were at 09:00 and 16:00 hours and before the chlorhexidine rinse. The toothpastes were rinsed as 3 g/10 ml water slurries and water as a 10 ml rinse each for 2 min. On day 5, subjects were scored for tooth and tongue stain intensity and area, and the product of these was calculated. The washout period was at least 9 days.

Results: Treatment differences for the teeth were highly significant but not for the tongue. Paired contrasts for tooth stain intensity, area and product were mostly all significantly in favour of reduced staining by the experimental formulation compared with water and the whitening product. There were no significant differences between water and the whitening product.

Conclusions: Using a forced dietary staining method, the data support a tooth stain-inhibitory/-removal action for the experimental formulation, but not the whitening product.

Key words: chlorhexidine; clinical trial; extrinsic stain; tea; toothpaste

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A common cause of tooth discoloration is the accumulation of extrinsic stains on or in the acquired pellicle covering the dental hard tissues (for a review, see Watts & Addy 2001). The most common source of the chromogenic material is thought to be derived from the diet or habits involving the use of tobacco. Tooth staining by dietary substances, notably tea, coffee and red wine, can be enhanced by the oral use of cationic antiseptics such as chlorhexidine, and polyvalent metal salts including tin and iron (for a review, see Addy & Moran 1995). Most populations consider extrinsic tooth discoloration as unaesthetic and dental professionals expend considerable time and effort in removing staining from teeth. Presumably, as a result of a public demand for home-use products to improve tooth colour, a large number of so-called ''whitening'' toothpastes can be found in the marketplace. The formulation of these products

appears directed at the control of extrinsic stain, to return teeth to their natural colour. Few products contain ingredients that could change the natural colour through bleaching: those that do have been questioned for efficacy based on the concentration and duration of action of the ingredients (Sharif et al. 2000). Toothpastes formulated to control extrinsic tooth staining could achieve an effect through stain inhibition or removal and by chemical or physical means. Thus, common to most toothpastes is the presence of abrasives and detergents, both of which could remove stain (for reviews, see Davis 1980, Forward et al. 1997). Indeed, it was the side effect of tooth staining when very low or non-abrasive toothpastes came on the market that drew attention to the role of abrasives in toothpastes (for a review, see Fischman 1997). Other ingredients that could remove stain are polyphosphates, which chelate calcium and may influence pellicle thickness, and enzymes directed at the pellicle layer. Most research on the efficacy of toothpastes for stain removal has been conducted in vitro, where a large variation in effect was reported (Sharif et al. 2000). Stain inhibition, like plaque inhibition, could be interpreted as the prevention of stain buildup by the incremental removal of stain by the regular use of a product. In the true sense, stain inhibition has been little researched, presumably because there are few agents that can prevent stain uptake by pellicle. Polyvinyl pyrollidone (PVP), a chemical finding many uses in industry including in cosmetics, appears to possess stain-inhibitory properties. Such dental stain inhibition has been noted in studies in vitro and in vivo (Barnett et al. 1994, Claydon et al. 2001). Although a considerable number of whitening products are available, there is little published research as to efficacy based on classical blind, randomised, controlled clinical trials. A similar criticism can be made concerning the efficacy of most, but not all, in office- and home-use tooth bleaching products.

The aim of the present study was to compare an experimental toothpaste, containing ingredients that, predictably, would both inhibit and remove stain, with a marketed whitening toothpaste and water as the negative control. The model tested the chemical effects of the agents on a forced chlorhexidine/tea staining method without tooth brushing.

Material and Methods

Ethical approval for the study was granted by the United Bristol, Healthcare Trust, Ethics Committee. The study was designed and conducted to comply with the Guidelines for Good Clinical Practice (GCP). A total of 24 subjects were recruited to participate in a single-centre, single, examiner blind, randomised, three-treatment crossover design study, balanced for first-order carry-over effects. Subjects were provided with written information concerning the study and gave signed and witnessed consent to participate. To participate, subjects had to be healthy with no relevant medical or pharmacotherapy histories that might influence the conduct of the study. Volunteers were of either gender and aged 18 years or older and had at least 12 assessable teeth, excluding molars, and of which at least seven had to be incisors. Subjects were dentally and periodontally healthy without fixed or removable orthodontic appliances or removable prostheses and full coverage restorations on teeth in the scoring region of the upper and lower arches. Subjects were excluded if they used tobacco products, had aesthetic restorations, which could become discolored, or were already taking chromogenic oral products, such as chlorhexidine, or medications that could stain the dentition. Teeth or subjects were also excluded if they exhibited dental defects or intrinsic discoloration, which might interfere with grading the outcome measures of the stain intensity and area.

The study treatments were as follows:

1. Toothpaste marketed as a tooth-whitening product.*
2. An experimental tooth-whitening toothpaste formulation (now a marketed product†).
3. Water (negative control).

One week prior to the study and during the washout periods, subjects brushed their teeth with a standard toothpaste‡ and toothbrush§. The method used a modification of a previous protocol designed to force the development of extrinsic stain on teeth within a period of a few days using reciprocal rinses of chlorhexidine mouth rinse and tea. The modifications, decided as a result of laboratory experimentation and then modelling the clinical study in vitro, were to further accelerate the stain formation: this involved tripling the tea concentration and increasing the temperature of the tea rinses.

After screening and enrolment, and during washout periods, the subjects were asked to perform tongue brushing up to the evening before day 1 of each of the 3, 4 day study periods. A professional dental prophylaxis was performed on each Friday before the Monday day 1 start of each period, to remove all plaque, stain and calculus from the teeth. On day 1 the teeth were re-examined to ensure they remained stain free. A further prophylaxis was performed if extrinsic staining was observed. An oral soft-tissue examination was also performed at the start of each period. Subjects then suspended normal tooth cleaning and commenced the allocated rinsing regimen, which differed only in which test agent was to be used. Thus, the test toothpaste or water rinses were performed at 09:00 and 16:00 h. To produce staining, subjects also rinsed eight times per day, approximately on the hour, from 09:00 to 16.00 h with a 10 ml volume of a 0.2% chlorhexidine rinse¶ followed by a 10 ml rinse of a black tea infusion at $50 \pm 3^{\circ}$C. Rinsing times for the interventions were 2 min and the chlorhexidine and tea 1 min. At times when the chlorhexidine tea rinsing coincided with the interventions (09:00 and 16:00 h), the interventions were rinsed first. The test toothpaste rinses were made up as slurries of 3 g in 10 ml of water. Water rinses were used as 10 ml volumes. The standard tea solution was prepared as 3 g of tea leaves boiled in 100 ml of water for 2 min, strained through gauze to remove the tea leaves and the infusion maintained in vacuum flasks; infusions were replaced once the temperature dropped below 47°C. The preparation of the toothpaste slurries was shortly before use and out of site of the subjects. The pastes themselves were coded as A or B and the code breaker was kept in a locked cabinet. The rinsing of the interventions was

*Rembrandt Whitening, Den-Mat Corporation, Santa Maria, CA, USA.

†Aquafresh Multiaction Whitening, GlaxoSmithKline, Weybridge, UK.

‡Aquafresh Mild 'n' Minty, GlaxoSmithKline, Weybridge, UK.

§Aquafresh Flex, GlaxoSmithKline, Weybridge, UK.

¶Corsodyl, GlaxoSmithKline, Weybridge, UK.

supervised. The chlorhexidine and tea solutions were taken at the appropriate times to the subjects at their workplace within the Dental Hospital for immediate use. On day 5, the subjects returned to the clinic and an oral soft-tissue examination was again performed. Dental stain intensity and area were then scored from the buccal surfaces of the upper and lower incisor, canine and premolar teeth according to the criteria of the stain index described by Lobene (1968). Similarly, tongue stain intensity and area were scored according to the criteria of the same author (Lobene 1968). All parts of the index score 0–3 with intensity ratings being no stain, light, moderate and heavy, and area being no stain and then in one-third of the surface area. Any adverse events during the study were to be reported to study personnel and, if thought appropriate, subjects would be assessed by one of the clinical investigators. At each scoring visit, the examiner directly questioned subjects as to adverse events during individual periods and before conducting the oral soft-tissue examination. The same clinician (HP) conducted all examinations and scorings. The examiner was trained in the indices used by an experienced examiner and participated in two related studies as a second scorer prior to the present study to confirm comparability in scoring. All assessments were performed in the same dental unit under identical and mainly artificial lighting conditions. Immediately after scoring, subjects received a professional prophylaxis. A washout period of 9 days was allowed between each treatment period when subjects returned to normal oral hygiene and were encouraged to tongue brush.

Statistical methods

The primary outcome measure for the study was the mean product of dental stain intensity and area calculated for both the gingival crescent and body of tooth sites. The secondary outcome measure was the mean product of tongue stain intensity and area. The main analyses were based on analysis of variance modelled on the subject, period and treatment. Point estimates, 95% confidence intervals and p-values were calculated for differences between each pair of treatments. On account of the possible non-Gaussian distributional form, confirmatory non-parametric tests were also performed, namely Friedman two-way analysis of variance followed by Wilcoxon-matched pairs signed-ranks tests for paired comparisons.

Results

The recruited subject group comprised 15 females and nine males aged between 23 and 56 years (mean age 32 years). All the subjects satisfactorily completed the study, and the data set remained orthogonal and did not require adjustment for confounding effects of period or subject differences. The mean dental stain area and intensity scores and the product scores for the gingival crescent and body zones are shown in Table 1. Three-way analysis of variance revealed significant differences in all gingival crescent data for period, subject and treatment variables (p ranged from <0.05 to <0.001), and in body data for period and treatment (p ranged from <0.01 to <0.001). Subject differences only reached significance for body stain area ($p<0.01$). In terms of mean gingival crescent and body stain intensity, area and product were always less with the experimental toothpaste than water or the whitening toothpaste. Data for water and the whitening product were similar. Contrasts between pairs of treatments for both gingival and body stain areas, intensity and product are shown in Tables 2 and 3. In every contrast, the experimental formulation resulted in significantly reduced stain compared with water and the whitening toothpaste (p ranged from <0.01 to <0.001). The confirmatory non-parametric p-values were less strong, but still revealed significant differences in favour of the experimental paste (p ranged from <0.05 to <0.001). There were no significant differences for tooth stain scores for any of the paired data comparisons of water and the whitening toothpaste.

Table 1. Summary statistics for stain intensity, area and product by treatment: gingival crescent and body of teeth

Treatment	Gingival crescent stain intensity	Gingival crescent stain area	Gingival crescent stain product
1. Experimental			
mean	2.13	1.85	4.49
N	24	24	24
SD	0.61	0.71	2.24
2. Whitening product			
mean	2.54	2.55	6.71
N	24	24	24
SD	0.49	0.55	2.07
3. Water			
mean	2.53	2.55	6.71
N	24	24	24
SD	0.46	0.46	1.90
Total			
mean	2.40	2.31	5.97
N	72	72	72
SD	0.55	0.66	2.30
Treatment	**Body stain intensity**	**Body stain area**	**Body stain product**
1. Experimental			
mean	1.16	1.03	1.85
N	24	24	24
SD	0.76	0.68	1.46
2. Whitening product			
mean	1.71	1.41	2.96
N	24	24	24
SD	0.80	0.55	1.73
3. Water			
mean	1.86	1.53	3.28
N	24	24	24
SD	0.65	0.49	1.83
Total			
mean	1.57	1.32	2.70
N	72	72	72
SD	0.79	0.61	1.77

Table 2. Contrasts between pairs of treatments for gingival crescent stain intensity, area and product measures

	Point estimate	95% confidence interval	*p*-value	*p*-value from Wilcoxon test
Intensity				
whitening versus experimental	+0.41	+0.18 to +0.64	0.001	0.009
water versus experimental	+0.40	+0.17 to +0.63	0.001	0.003
water versus whitening	−0.01	−0.25 to +0.22	0.90	0.82
Area				
whitening versus experimental	+0.70	+0.45 to +0.94	<0.001	0.001
water versus experimental	+0.71	+0.46 to +0.95	<0.001	<0.001
water versus whitening	+0.01	−0.24 to +0.25	0.96	0.68
Product				
whitening versus experimental	+2.22	+1.31 to +3.14	<0.001	0.003
water versus experimental	+2.22	+1.31 to +3.13	<0.001	<0.001
water versus whitening	−0.004	−0.92 to +0.91	0.99	0.75

Table 3. Contrasts between pairs of treatments for body of tooth stain intensity, area and product measures

	Point estimate	95% confidence interval	*p*-value	*p*-value from Wilcoxon test
Intensity				
whitening versus experimental	+0.55	+0.19 to +0.90	0.004	0.023
water versus experimental	+0.70	+0.34 to +1.05	<0.001	0.002
water versus whitening	+0.15	−0.20 to +0.51	0.40	0.63
Area				
whitening versus experimental	+0.38	+0.12 to +0.64	0.005	0.040
water versus experimental	+0.49	+0.23 to +0.75	<0.001	0.006
water versus whitening	+0.11	−0.14 to +0.37	0.38	0.81
Product				
whitening versus experimental	+1.11	+0.28 to +1.94	0.010	0.027
water versus experimental	+1.42	+0.60 to +2.25	0.001	0.004
water versus whitening	+0.31	−0.51 to +1.14	0.45	0.72

Table 4. Summary statistics for tongue stain intensity, area and product by period and treatment

Treatment	Tongue stain area	Tongue stain index	Tongue stain product
1. Experimental			
mean	1.92	2.04	4.83
N	24	24	24
SD	1.10	1.08	3.53
2. Whitening product			
mean	2.17	2.25	5.42
N	24	24	24
SD	0.92	0.90	3.26
3. Water			
mean	2.33	2.33	5.92
N	24	24	24
SD	0.87	0.82	2.96
Total			
mean	2.14	2.21	5.39
N	72	72	72
SD	0.97	0.93	3.24

The mean stain area, intensity and product for the tongue are shown in Table 4. Three-way analysis of variance revealed that subject differences were significant ($p<0.001$), but with the exception of period differences for intensity ($p<0.05$) period and treatment differences did not reach significance ($p>0.05$). Paired treatment comparisons were therefore not deemed appropriate.

No adverse events were reported or noted by the clinical examiner.

Discussion

This model was primarily conceived to study chemical stain control by toothpastes. While it is true that the actual efficacy of a product cannot be fully understood without the concomitant use of a toothbrush, it is likely that all toothpastes through abrasive systems will remove stain from surfaces contacted during the brushing cycle (Davis 1980). As with chemical plaque control however, ''whitening'' toothpastes contain chemicals to control stain at sites difficult to access or missed by the toothbrush. In any study, evaluating the effects of treatments on an outcome measure, the magnitude of the outcome measure developing under the control treatment will influence whether significant differences can be shown. This must certainly apply to extrinsic dental stain, which, under normal conditions, develops slowly. Even enhancing dietary staining with chlorhexidine reveals considerable subject variation, although using a crossover design should control for this phenomenon. The forced stain model, as employed previously (Addy et al. 1991), was modified to encourage high amounts of staining in the placebo control group. The modification manipulated two variables in the standard model and the effects were studied in vitro. Thus, the strength and the temperature of the tea were found to influence markedly the rate of stain development in vitro (unpublished data) and were applied in this study in vivo.

The results clearly demonstrated that, for tooth stain, the test formulation inhibited chlorhexidine/tea staining to a significantly greater extent than the placebo, water and the commercial whitening toothpaste. Interestingly, and perhaps surprisingly, there were no differences in stain inhibition between the water control group and the commercial whitening toothpaste. In controlling extrinsic tooth discoloration, it is apparent that whitening toothpastes could exert one or both the actions, namely stain inhibition and stain removal. The experimental formulation contained ingredients to exert both effects. Thus, the contained PVP was shown to inhibit chlorhexidine/tea staining both in vitro and in vivo (Barnett et al. 1994, Claydon et al. 2001), whereas the sodium lauryl sulphate detergent would be expected to remove stain (Addy et al. 1991). The role of other ingredients in the formulation also

probably played a role, although whether the action is inhibitory, removal or both is unclear. The contained sodium tripolyphosphate (STP) could exert actions both on the pellicle or on the stain to inhibit or remove stain, respectively. Data from studies in vitro suggest that stain removal is the more dominant effect of STP since toothpastes containing the combination of polyphosphates and sodium lauryl sulphate were more effective than other formulations without polyphosphates such as STP (Sharif et al. 2000). One could postulate that the action of the test formulation was merely to inhibit the chlorhexidine/tea staining mechanism by inactivation of the chlorhexidine by toothpaste ingredients (Barkvoll et al. 1989, Owens et al. 1997, Sheen et al. 2001) and may therefore not influence natural stain. This seems unlikely since staining associated with chlorhexidine appears to be an exaggeration of natural stain: the chlorhexidine acting to attract the chromogens ionically onto the tooth surface (for a review, see Addy & Moran 1995). Also, the frequency, eight times, of chlorhexidine tea rinsing per day would be expected to outweigh any chlorhexidine-inhibitory action of toothpaste. Indeed, if a toothpaste chlorhexidine-inhibitory action only had occurred, significantly reduced staining by the commercial whitening product compared with water should have been seen.

The results for the commercial product were disappointing, although there are few controlled clinical studies on such whitening products by which to evaluate claims of efficacy. Certainly, the product contains ingredients, which might inhibit or remove stain but, in the event, their actions appeared insufficient or too slow to provide clinically meaningful data. This would be consistent with a study in vitro, which showed little stain removal effects of this particular formulation.

In conclusion, the experimental formulation was significantly effective in reducing extrinsic dental stain, and within the present model was probably achieved by both chemical stain-inhibitory and removal processes. The model could be applied for all ''whitening toothpastes'' to bridge what is a dearth of information on the actual efficacy of such products.

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