The chemical stain removal properties of 'whitening' toothpaste products: studies *in vitro*

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Background A considerable number of toothpastes are available as tooth whitening products. Most appear to contain ingredients that might remove extrinsic stains rather than change natural tooth colour. Extrinsic stain removal could be achieved by physical or chemical means.

Aim The purpose of this study was to measure the chemical stain removal properties of a range of whitening toothpaste products and experimental formulations using a standardised method *in vitro.*

Materials and method 5 separate studies were conducted involving a total of 39 agents of which 28 were whitening products, 7 were experimental formulations, 2 were oxidising mouthrinses used as positive controls, 1 was a popular fluoride toothpaste product as a benchmark control, and 1 was water as the negative control. The formulations and controls varied in each study. The stain model was saliva/chlorhexidine/tea stain developed on optically clear acrylic to an optical density of at least 2.0. Groups of stained specimens were exposed to standard slurries or solutions of each test agent for 1 minute periods up to 5 minutes. Optical density readings were taken at each 1 minute time point. Analyses were based on per cent stain remaining after 5 minutes and time to 75% stain remaining.

Results 3 toothpaste products achieved 100% stain removal by 5 minutes; 2 of these in 3 out of 4 studies in which they were used. 4 experimental formulations also achieved 100% stain removal. In general agents with high total stain removal also had short times to 75% stain remaining. The majority of agents tested had low total chemical stain removal and prolonged times to 75% stain remaining. A few agents were little different from water and several similar in effect to the conventional fluoride toothpaste. This method *in vitro* tests agents under the best case scenario conditions for chemical stain removal.

Conclusion Only a small number of the whitening toothpaste products have good chemical stain removal potential; the majority are unlikely to achieve their claimed benefits through chemical stain removal. There is clearly a need for further data on the actual effects of such products using both methods *in vitro* and particularly *in vivo* or *in situ.*

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The natural colour of permanent teeth is largely determined by the dentine, modified by the thickness and translucency of the overlying enamel. Discoloration of teeth can occur because of the deposition of a variety of pigments into or onto the tooth. Such causes of discoloration are usually classified as intrinsic or extrinsic staining respectively; signifying the source of the stain.[1–3] Clearly, some extrinsic stains can become internalised through enamel defects or cracks or as a result of dentine becoming exposed.[2,3] Most extrinsic stains appear to be deposited on or in the acquired pellicle. The aetiology of such discoloration is poorly understood but compounds within the diet or from tobacco are considered the most common chromogens.[4]

Furthermore, the dental staining associated with the cationic antiseptics and polyvalent metal salts appears to arise from an interaction and precipitation reaction with dietary chromogens.[2,5,6] Attempts to improve the colour of teeth could be directed against either intrinsic or extrinsic stains, or by changing the natural colour of the teeth. To date changing the natural colour of teeth or removing intrinsic discoloration has relied mainly on bleaching agents such as carbamine peroxide. Reports of success appear largely drawn from laboratory studies or anecdotal or case report data, usually relating to the use of professionally delivered bleaching systems.[7,8] At present there is a dearth of classical blind randomised controlled clinical trials on such systems. Similarly, despite the recent appearance in the market place of a considerable number of home use tooth 'whitening' products, mainly toothpastes, there is little clinical evidence as to their comparative efficacy. Most such products contain ingredients that would be expected to remove extrinsic stains rather than bleach teeth. Although a few products do contain bleaching agents, the permitted level appears far too low to have any effect, particularly given the very short contact time with teeth during normal brushing.

Extrinsic stain removal from teeth by toothpaste could be achieved by physical means through abrasives or chemical means through organic solvents, notably detergents.[9,10] Since most toothpastes, whitening or otherwise, contain abrasives capable of removing organic stain, it is the chemical agents which offer potential benefit, particularly to sites not easily accessed by the toothbrush. The aim of this study was to compare, in a series of experiments *in vitro,* the chemical stain removal properties of commercial tooth whitening toothpaste products and experimental formulations. The method used a previously reported reproducible stained substrate by which to measure optically stain removal.[11]

Method and materials

The method involves chlorhexidine enhanced tea staining of optically clear methyl methacrylate (Perspex) specimens. Acrylic specimens measuring 30x10x5mm were cut to fit the specimen chamber of a UV/visible spectrophotometer. Staining was produced by

cycling specimens through human saliva for 2 minutes, a 0.2% chlorhexidine mouthrinse for 2 minutes and a standard tea solution for 60 minutes. The whole staining process was then repeated. A brief rinsing in deionised water was performed after each component of the cycle. The treatment cycle was repeated until the optical density of the specimens was > 2.0. The standard tea solution was produced by boiling 10 gm of tea leaves in 1 litre of water for 2 minutes. The infusion was filtered through gauze to remove the tea leaves and then allowed to cool to room temperature. All experiments were performed at room temperature 20±3°C.

Five experiments were performed over an approximate 2-year period involving a collection of toothpaste products, described as whitening toothpastes, which were purchased from retail outlets or provided by a third party. One study also involved a whitening gel. Two studies included a conventional fluoride toothpaste product and three studies included experimental formulations. Three studies used water as a negative control; and three studies, one of two oxygenating mouthrinses based on peroxyborate or peroxycarbonate. These were considered as positive controls because of their known effect on the stain model and for peroxyborate an effect on stain *in vivo*.[12] It should be noted that experimental formulations referred to in this report were experimental at the time of the particular experiment. It is possible that since the time of a particular experiment some of the experimental formulations may have become products. However, since the authors could not be certain of the final formulation of a new product, to avoid error, these formulations remain designated experimental. For all toothpastes and the gel a standard slurry was prepared by thoroughly mixing 3 gm toothpaste/gel in 10 ml water. In the case of the oxygenating mouthrinses the powdered contents of the sachets were dissolved in 15 ml water. Water was used in 15 ml volumes. All treatments were used immediately after preparation. Stained acrylic specimens, of known baseline optical density, were randomly allocated to each treatment. In study 1, a total of three specimens per treatment were allocated, whereas in studies 2, 3, 4 and 5 a total of six specimens were allocated. Specimens in individual screw cap bottles were tumbled in the test slurry/solution for 1 minute, rinsed briefly in water, bench dried and the optical density read on the spectrophotometer. This procedure was repeated a further four times giving a total exposure time of 5 minutes of specimens to the test formulation.

Statistical methods

The progression of stain removal for each specimen was characterised in two ways. First, the percentage stain remaining at each of 1 to 5 minutes, Y1 to Y5, was calculated. Second, the estimated times to 75%, 50% and 25% stain remaining, T75, T50 and T25, were calculated by interpolation. A square root transformation of scale was used because the rate of stain removal slowed as removal progressed. The interpolation used the lowest value reached by each time point in the event of non-monotonic behaviour. Y5 and T75 were chosen as the most informative outcome measures because many formulations produced little effect. For each of the five studies separately one-way analyses of variance were

Table 1 Formulations used in the five stain removal experiments together with manufacturer/retailer

Formulation	No	Manufacturer/Retailer	Study number				
Aquafresh Whitening	1	SmithKline Beecham UK	1		3		5
Arm & Hammer Extra Whitening	2				3		
Asda Whitening	3	Asda Stores, UK				4	
Beverley Hills Natural White	4	Purity Labs, UK		2			
Beverley Hills Natural White Sensitive	5	Purity Labs, UK	1		3		
Beverley Hills Natural White Baking Soda	6	Purity Labs, UK	1				
Boots Sensitive Whitening	7	Boots Company, UK	1				
Boots Total Care Whitening	8	Boots Company, UK	1				
Boots Advance Whitening	9	Boots Company, UK			3		
Boots Advance Whitening Baking Soda	10	Boots Company, UK					5
Colgate Regular	11	Colgate Palmolive, UK		2			
Colgate Platinum	12	Colgate Palmolive, UK	1	2	3		5
Colgate Whitening	13	Colgate Palmolive, UK					5
*Dectrapharm Superwhite	14	Dectrapharm, Germany		2			
Janina	15	Janina Int. UK			3		5
Macleans Whitening	16	SmithKline Beecham, UK		2	3		
Pearl Drops Original (blue)	17	Carter Wallace, UK					5
Pearl Drops (pink)	18	Carter Wallace, UK				4	
Rapid White Toothpaste	19	Natural White, USA			3		5
Rembrandt (white)	20	Den Mat Corp. USA	1	2	3		
Rembrandt Original (green)	21	Den Mat Corp. USA					5
*Super White	22	Dectra Pharm, Germany		2			
Superdrug Sensitive Whitening	23	Superdrug Stores, UK	1				
Superdrug Ultracare	24	Superdrug Stores UK	1			4	
Tesco Whitening	25	Tesco Stores, UK	1			4	
Theramed	26	Henkel Cosmetics, Germany			3		
Topal Plus	27	DEP Corp, USA				4	
Yotuel Whitening Toothpaste	28	Biocosmetics, Spain				4	5
Yotuel Whitening Gel	29	Biocosmetics, Spain					5
Unmarketed agents in development	30	Richard Brierley, Ireland					5
	31	Richard Brierley, Ireland					5
	32	Richard Brierley, Ireland		2			
	33	Richard Brierley, Ireland		2			
	34	Richard Brierley, Ireland					5
	35	Richard Brierley, Ireland					5
	36	Richard Brierley, Ireland					5
Water	37		1			4	5
Kavosan	38	Oral B, Germany	1				5
Bocasan	39	Oral B, UK		2			

*These two products were supplied in different packaging and data was collected and analysed separately as if different formulations

Table 2 Mean and standard deviation of per cent stain remaining after 5 minutes (Y5) exposure to the test formulations

Formulation number	Formulation	Mean	Standard deviation	Specimen number
Study 1				
1	Aquafresh Wh	0.0	0.0	3
5	Bev Hills NW Sens	51.4	12.2	3
6	Bev Hills NW BS	31.1	4.3	3
7	Boots Sens Wh	70.2	19.5	3
8	Boots TC Wh	58.8	5.8	3
12	Colgate Plat	33.5	5.4	3
20	Rembrandt	71.5	9.9	3
23	Superdrug Sens Wh	39.0	3.0	3
24	Superdrug Ultra	56.7	18.2	3
25	Tesco Wh	30.4	10.0	3
37	Water	91.3	10.6	3
38	Kavosan	10.6	1.4	3
Study 2				
16	Macleans Wh	6.4	2.2	6
4	Bev Hills NW	71.0	5.1	6
11	Colgate Reg	63.1	6.9	6
12	Colgate Plat	66.8	4.6	6
14	Dectrapharm	25.9	3.8	6
20	Rembrandt	86.9	4.1	6
22	Super White	17.0	2.5	6
32	Experimental	4.4	1.3	6
33	Experimental	37.4	2.8	6
39	Bocasan	1.3	0.2	6
Study 3				
16	Macleans Wh	0.0	0.0	6
1	Aquafresh Wh	0.0	0.0	6
2	3 Arm & Hammer	36.5	13.2	6
5	Bev Hills NW Sens	6.7	6.3	6
9	Boots Adv Wh	30.1	5.5	6
12	Colgate Plat	60.4	6.8	6
15	Janina	65.7	10.6	6
19	Rapid White Paste	19.5	5.5	6
20	Rembrandt	78.0	8.5	6
26	Theramed	38.8	12.5	6
Study 4				
3	Asda Wh	68.0	6.7	6
18	Pearl Drops	63.9	9.1	6
24	Superdrug Ultra	52.1	6.9	6
25	Tesco Wh	63.1	9.9	6
27	Topal Plus	37.2	7.8	6
28	Yotuel Wh Paste	58.4	9.7	6
37	Water	71.5	11.0	6
Study 5				
1	Aquafresh Wh	14.9	13.4	6
10	Boots Adv Wh BS	0.0	0.0	6
12	Colgate Plat	63.6	7.9	6
13	Colgate Wh	56.9	11.3	6
30	Experimental	0.0	0.0	6
15	Janina	62.8	9.9	6
17	Pearl Drops Orig	71.0	11.3	6
31	Experimental	0.0	0.0	6
19	Rapid White	69.3	9.8	6
21	Rambrandt Orig	66.8	5.6	6
28	Yotuel Wh Paste	71.9	11.5	6
29	Yotuel Wh Gel	74.1	6.1	6
34	Experimental	0.2	0.4	6
35	Experimental	51.4	5.6	6
36	Experimental	64.3	12.7	6
37	Water	71.5	11.0	6
38	Kavosan	5.9	2.7	6

performed to compare Y5 and T75 between all formulations used in that study. Confirmatory non-parametric (Kruskal-Wallis) ANOVAs were performed because distributional form was not close to Gaussian. These analyses revealed large differences between formulations and, at such a level of significance that, multiple comparison problems were considered only a hypothetical caveat. The final analysis fitting together evidence from the five studies was 2-way ANOVA modelling on study and agent. This was done for each of eight outcomes Y1 to Y5 and T75, T50 and T25. Treatment contrasts specified as of prior interest were constructed comparing each of the formulations 1 and 16 (Table 1) with each of the others. Confidence intervals as well as *P*-values were calculated, but are not shown in detail.

Results

The data set comprised figures for per cent stain remaining at five time points for five studies involving a total of 39 treatments, together with time to stain remaining for 75%, 50% and 25%. However as stated, the most appropriate results for presentation were considered the 5 minute end point (Y5) and the time to 75% remaining (T75). Nevertheless where appropriate reference to other analyses will be made but without the presentation of the raw data. The means and standard deviations of the per cent remaining stain after exposure to the individual formulations in the five studies at the 5 minute time points are shown in Table 2. In each study, there are extreme differences in the percentage of stain removed. Thus, products 1 and 16 consistently approach 100% stain removal in studies 1, 2 and 3, and 85% for product 1 in study 5. Only two other marketed products, 5 and 10, achieve or approach similar results in individual studies. Interestingly, experimental formulations 30, 31, 32, and 34 also approach or achieve 100% stain removal. In most instances all of these treatments equal or better the positive control oxidising mouthrinse products, 38 and 39. Only two other products 19 and 22 removed just over 80% of the stain. At the other extreme most treatments did not achieve 50% stain removal and in mean terms some were little different from water.

The mean and standard deviation of the time to 75% stain remaining T75 for individual treatments in the five studies are shown in Table 3. Essentially products and experimental formulations 1, 10, 16, 30, 31 and 34 which had high stain removal efficacy also had very short T75 times. However, a number of other products also had relatively short T75 times but poor Y5 total stain removal. This would be consistent with the non-monotonic stain removal pattern whereby for many products stain removal slowed, in some cases dramatically as exposure time increased. Thus, most T75 times ranged from 2 to 5 minutes, with a number of T75 times little different from water. Some products clearly did not even achieve a T75 time point and are listed as 5 minutes.

Analysis of variance for Y5 and T75 showed highly significant differences between treatments in each of the five experiments ($P < 0.001$). This was confirmed by non parametric tests (study 1, P values 0.001 and 0.002 respectively and < 0.001 for all other studies). Analysis of variance for each of the eight outcomes, fitting together evidence from the five studies, demonstrated also highly significant differences between treatments and between studies ($P < 0.001$). Using product 16 for comparison with other products across the five studies, at 1 minute there was a highly significant difference between 16 and each other product (P ranged from < 0.01 to < 0.001) except product 1 ($P > 0.05$). With the exception of formulations 10, 30, 31, 34, 38 and 39 differences were in favour of product 16. However, these formulations consisted of the two positive control oxidising mouthrinses and three experimental formulations. Only product 10 performed better than product 16 and only in one experiment. Similar results were obtained when product 1 was used as the comparator. At 2 to 5 minutes and stain remaining times T75, T50 and T25, the pattern remains the same

Table 3 Mean and standard deviations of the time (minutes) to 75% stain remaining (T75) for each test formulation.

Formulation number	Formulation	Mean	Standard deviation	Specimen number
Study 1				
1	Aquafresh Wh	0.18	0.01	3
5	Bev Hills NW Sens	1.86	0.26	3
6	Bev Hills NW BS	1.29	0.21	3
7	Boots Sens Wh	3.55	2.51	3
8	Boots TC Wh	2.50	0.99	3
12	Colgate Plat	0.80	0.44	3
20	Rembrandt	4.32	1.18	3
23	Superdrug Sens Wh	0.72	0.14	3
24	Superdrug Ultra	1.63	0.73	3
25	Tesco Wh	0.93	0.57	3
37	Water	5.00	0.00	3
38	Kavosan	0.23	0.01	3
Study 2				
16	Macleans Wh	0.29	0.05	6
4	Bev Hills NW	3.40	1.66	6
11	Colgate Reg	2.99	1.04	6
12	Colgate Plat	2.75	0.82	6
14	Dectrapharm	0.87	0.09	6
20	Rembrandt	5.00	0.00	6
22	Super White	0.79	0.11	6
32	Experimental	0.61	0.16	6
33	Experimental	1.73	0.20	6
39	Bocasan	0.16	0.00	6
Study 3				
16	Macleans Wh	0.24	0.03	6
1	Aquafresh Wh	0.15	0.02	6
2	Arm & Hammer	1.64	0.44	6
5	Bev Hills NW Sens	1.31	0.68	6
9	Boots Adv Wh	0.92	0.19	6
12	Colgate Plat	2.30	0.55	6
15	Janina	3.65	0.68	6
19	Rapid White Paste	0.77	0.18	6
20	Rembrandt	4.29	1.69	6
26	Theramed	2.08	0.26	6
Study 4				
3	Asda Wh	3.24	1.19	6
18	Pearl Drops	3.71	0.79	6
24	Superdrug Ultra	1.61	0.15	6
25	Tesco Wh	2.15	0.58	6
27	Topal Plus	1.00	0.17	6
28	Yotuel Wh Paste	3.53	0.90	6
37	Water	3.84	1.88	6
Study 5				
1	Aquafresh Wh	0.70	0.11	6
10	Boots Adv Wh BS	0.25	0.03	6
12	Colgate Plat	2.73	1.49	6
13	Colgate Wh	2.43	1.06	6
30	Experimental	0.23	0.02	6
15	Janina	3.06	1.00	6
17	Pearl Drops Orig	4.07	0.89	6
31	Experimental	0.19	0.04	6
19	Rapid White	3.04	1.93	6
21	Rembrandt Orig	2.80	1.47	6
28	Yotuel Wh Paste	3.60	1.73	6
29	Yotuel Wh Gel	3.59	1.30	6
34	Experimental	0.25	0.03	6
35	Experimental	1.59	0.68	6
36	Experimental	3.04	1.91	6
37	Water	3.84	1.88	6
38	Kavosan	0.21	0.02	6

for differences between products 1 and 16 and the remaining products. Some significant differences of product 16 over product 1 were seen at 2 and 3 minutes and T25. Also experimental formulation 32 at 3 minutes became better than product 1.

Finally, an alternative and simple approach to appraising the data is to define success. Interestingly within each study for each treatment, the proportion of specimens (3 or 6) reaching 50% stain removal by 1 minute (Y1) was either 100% or more usually 0%. Those products excluding experimental formulations and the positive controls, achieving the 100% success were: study 1, product 1; study 2, product 16; study 3, product 1 and 16; study 4, none; study 5, product 10. In summary, the picture overall showed that products 1 and 16 generally outperformed all other agents evaluated. Product 10 and some of the experimental formulations also performed very well.

Discussion

This stain formation/stain removal model has been used for many years in dental research.[11,13] Originally, the method was developed to study extrinsic staining associated with cationic antiseptics and polyvalent metal salts.[6,13] Later the model was extended to measure chemical stain removal by toothpastes.[11] Acrylic is employed since it is a common dental material which appears to stain in a similar manner to enamel, but has the advantage of allowing accurate optical density readings to be obtained. The method has been used in clinical studies to both investigate stain formation and removal *in vivo*.[12,14,15] In the present *in vitro* study, the method allows the potential of agents to chemically remove organic stain, from a substrate surface, to be measured. The laboratory environment is almost certainly a best case scenario for agents to work within for a number of reasons. The stain developed is almost entirely organic representing discoloration which would develop initially *in vivo*. There is no potential for calcification of the stain which occurs in vivo, particularly under the influence of chlorhexidine,[16] thereby impeding the action of many stain removing formulations. The contact of agent with stain is total and highly standardised and controlled, thereby facilitating comparative studies. Slight baseline variations in stain clearly occur but these can be adjusted for by appropriate statistical methods or, as here, by calculating percent changes from baseline. The limitation of the method is that it purposely avoids mechanical effects of brushing, since virtually all toothpastes have the potential for stain control through abrasion.[9,10] Indeed, tooth staining has been the consequence of using low or non abrasive toothpastes.[9]

The data bank was collected over a period of 2 years as new products came to the market or requests to assess experimental formulations arose. In an ideal study design, all formulations of interest would be tested together and possibly the experiment repeated several times. This however would be logistically difficult if not impossible. Differences thus existed between studies, irrespective of differences in products tested. These could relate to the stain developed, being affected by differences in saliva used, examiner, experimental error or variation. Nevertheless, the data were surprisingly consistent within formulations, used in more than one study and more particularly between formulations when comparisons of efficacy were made. Only a very small number of actual products approached complete stain removal within the test period and of these, four products were equal to or better than the positive control oxidising mouthrinses. Interestingly, some experimental formulations were similarly effective to the most effective products. However, the present authors cannot be totally certain as to the final fate of these experimental formulations. Therefore, it would be unsafe to extrapolate findings here to products now available or planned for launch which are based on the experimental formulations.

The study is a formulation or product comparison and not one of specific ingredients. Toothpastes have complex and variable

recipes[10] and experimental formulations would have to be developed and tested to determine the specific effects of any one ingredient. It was in part for this reason that specific actives in products were not listed. In many cases the likely actives are not stated and many ingredients common to all toothpastes can remove stain, notably detergents. With these points in mind, it is clear that in this model the majority of whitening toothpaste products performed poorly in terms of chemical stain removal. Moreover, the data indicated that many products removed some stain within 1–2 minutes and little thereafter. It is difficult therefore not to conclude that in the more demanding oral environment, where the product is in contact with individual teeth for relatively short periods, most of these products would not benefit tooth colour through a chemical stain removal process. This is despite the considerable comparative cost of many whitening products. Only a small number of products appear to offer a benefit to the user of tooth whitening through stain control. Clearly, some products could influence tooth colour by changing the natural colour of teeth. This essentially involves bleaching through enamel which appears unlikely for toothpastes. Most do not have bleaching agents within the formulation and those that do have contact times too short and levels of bleach too low to have effect. With so many and relatively very expensive cosmetic toothpastes available there is a need for confirmatory data from both laboratory and clinical studies to support the implied claims of the products.

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