Dietary staining \textit{in vitro} by mouthrinses as a comparative measure of antiseptic activity and predictor of staining \textit{in vivo}

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ABSTRACT
Extrinsic staining of teeth is a side-effect of some antiseptic mouthrinses. However, few of the many rinse products available to the general public have been investigated for their propensity to cause staining. Dietary factors play an aetiological role in staining and have been used \textit{in vitro} to study and compare the activity of rinses. The aim of this study was to assess rinse products for staining \textit{in vitro} and, through the staining reaction, to compare the activity of products containing the same ingredients. Perspex blocks, with or without saliva pretreatment, were soaked in rinses for 2 min, washed and placed in a standard tea solution for 60 min and then the optical density (OD) read on a spectrophotometer. The cycle was repeated 10 times for saliva and 17 times for no saliva specimens or until the maximum OD was exceeded. A series of three separate experiments was performed by this method. The maximum OD was not exceeded by any product before seven passages and therefore data were compared at six passages. For most products OD increased with saliva pretreatment. Some cetylpyridinium chloride (CPC) rinses stained comparably to a chlorhexidine rinse. CPC rinses, most of which contained the same concentration of the antiseptic, varied considerably in their propensity to induce staining and one was little different to water controls. A 0.1% chlorhexidine rinse stained slightly more than a 0.2%. A phenolic/essential oil product produced some staining but zinc, triclosan and other essential oil rinses did not stain. Consistent with clinical data, tea staining \textit{in vitro} can be used to indicate the propensity of rinses to stain \textit{in vivo}, however extrapolating the data to the clinic must take into account the substantivity of different active agents. Also, the model can be used to evaluate the availability of certain ingredients, and therefore the activity of the product.


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INTRODUCTION
Extrinsic staining of the teeth is a recognized local side-effect of the use of cationic antiseptic mouthrinses, particularly chlorhexidine\textsuperscript{1,2}. Indeed, the absence of extrinsic tooth staining with chlorhexidine products may be indicative of lack of efficacy, due to reduced availability of the antiseptic in a formulation\textsuperscript{3–5} or failure in compliance on the part of the patient. The mechanism by which cationic antiseptics produce tooth staining is a matter for debate\textsuperscript{6}, however there is no doubt that agents such as chlorhexidine and cetylpyridinium chloride (CPC) will precipitate or bind to surfaces food dyes and dietary chromogens \textit{in vitro}\textsuperscript{7–13}. Several clinical studies also strongly support a dietary aetiology for staining associated with cationic antiseptics, and certain beverages are particularly chromogenic \textit{in vivo}\textsuperscript{12,14}. A similar dietary interaction mechanism appears to explain the tooth staining associated with certain metal salts, notably iron and tin\textsuperscript{13,16}.

In many countries there are at present a large number of 'over the counter' and/or prescription antimicrobial mouthrinses. Surprisingly, there has been little interest in the potential of such products to cause extrinsic tooth staining. Yet for some products, notably those containing CPC, a staining side-effect would be expected\textsuperscript{2,17,18}, and for others such as phenolic rinses, a staining side-effect
has been alluded to, and supported by some clinical data. This study in vitro was based upon the known potential of some antiseptics to interact with and bind dietary chromogens onto surfaces. The study had three aims. First, to compare the ability of a number of antiseptic mouthrinse products to bind chromogenic material from tea to acrylic surfaces as a possible predictor of staining in vivo. Secondly, to compare the staining potential of different CPC-containing mouthrinse products as an indicator of antiseptic availability and, therefore, product efficacy in vivo. Thirdly, to compare two chlorhexidine mouthrinses, available in the UK, for staining potential.

METHOD AND MATERIALS

The study in vitro employed essentially the same method described by Addy et al.. A standard tea solution was prepared each day by boiling 8 g of a branded tea in 800 ml of distilled water for 2 min. The solution was allowed to cool in a refrigerator at 4°C for 30 min and the infusion finally filtered through gauze to remove the tea leaves. Rectangular blocks of optically clear acrylic Perspex, ICI, Macclesfield, UK measuring 30 mm X 10 mm X 5 mm were prepared to fit the specimen chamber of a UV/visible double beam spectrophotometer.

The mouthrinse products, together with the contained antiseptic or other active ingredient, used in the experiments were as follows (the letters in bold are the abbreviations used in the result bar charts. Figs 1-4): (1) Listermint with Fluoride (CPC, NaF). LF; (2) Sainsbury's Antiseptic Mouthwash with Fluoride (CPC, NaF). SAS; (3) Tesco Dental Care Original (CPC, NaF). T; (4) Sainsbury's Oral Health Mouthwash, Antiplaque Formula with Fluoride (CPC, NaF). SH; (5) Sainsbury's Oral Health Extra Strength Antiseptic Mouthwash (CPC, Essential Oils). SES; (6) Corsodyl (Chlorhexidine), Cors; (7) Listermint Antiseptic Mouthwash (CPC). L; (8) New Reach Anti-Plaque Formula Mouthwash (CPC, NaF), Rch; (10) Mentadent P-Gum Health Mouthwash (Zinc, NaF), Ment; (11) Listerine Antiseptic Mouthwash (Phenol/Essential Oils). List; (12) Tesco Dental Care with Fluoride (CPC, NaF). TF; (13) Boots Freshmint Anti-Plaque Formula with Fluoride (CPC, NaF). B; (14) MacLeans Active Mouthguard with Fluoride (CPC, NaF), Mac; (15) New Colgate Actibrush (Triclosan, NaF), Act; (16) Tom's Natural Mouthwash (Aloe Vera, Essential Oils). Tom; (17) Superdrug Oral Health Mouthwash with Fluoride (CPC, NaF). SD; (18) Oraldene (Hexitetidine). Oral; (19) Scrarch Dental Rinse (CPC). Sch; (20) Eludril (Chlorhexidine); (21) 0.1% CPC, 0.1%; (24) 0.05% CPC, 0.05%; (25) Water, W.

All products were newly purchased from a suitable retail outlet immediately before commencing the study. Water was used as the negative control. Groups of six blocks were allocated to each rinse product and then subdivided into two sets of three blocks. One set was placed into pooled human saliva for 2 min and the other into distilled water for 2 min. Sets were removed from these preproduct solutions and washed in water before being placed into individual universal containers filled with the assigned rinse product. After 2 min the blocks were removed, washed in distilled water and placed into universal containers, filled with the tea solution, for a period of 60 min. The blocks were then removed from the tea, briefly washed in distilled water and allowed to air dry. Finally, the optical density of the blocks was read on the spectrophotometer at the lambda maximum for tea of 395 nm. The instrument was zeroed using untreated blocks. The sequence was then repeated for 10 passages of the saliva-pretreated specimens, 17 passages of the water-pretreated specimens or until the maximum optical density reading (approximately 3) of the instrument was exceeded. In the latter case the previous optical density reading was taken as the end point of the experiment.

In view of the findings of this experiment it was felt necessary, as a confirmatory exercise, to repeat the study using a selected number of CPC-containing mouthrinse products and water as control (Study 2). For this experiment new products were purchased. Additionally, the experiment included freshly prepared aqueous solutions of 0.1% and 0.05% CPC. The methodology was the same as in the previous experiments.

Finally, the same methodology was used to record the staining developing on specimens exposed to the chlorhexidine products. Corsodyl (0.2%) and Eludril (0.1%), after 12 passages (Study 3). Only saliva-treated specimens were used and Eludril employed neat and diluted 1 in 3 as recommended by the manufacturer.

RESULTS

In Experiments 1 and 2 the maximum optical density reading of the instrument was exceeded after a different number of passages depending on saliva pretreatment and the particular rinse. However, no treatment exceeded the maximum optical density reading at six passages and data were compared at this number of passages and shown in Figs 1-3. Mean optical density readings for non-CPC rinses used in Experiment 1 are shown in Fig. 2. Data for the chlorhexidine rinses were compared after 12 passages and shown in Fig. 4.

Statistical analyses of differences between products were performed only for products which contained the same active ingredients, namely the CPC rinses used in Experiments 1 and 2 and the chlorhexidine rinses used in Experiment 3. The significance of differences between CPC rinses and effects of saliva was determined by parametric two-way analysis of variance after log transformation of the data and also for differences between rinses only by Kruskal-Wallis non-parametric analysis of variance. Multiple comparisons between pairs of CPC treatments were considered inappropriate. In Experiment 3 unpaired t tests compared with the paired contrasts of the three chlorhexidine rinses.
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Experiment 1

Fig. 1 shows the tea staining on saliva and no saliva treated blocks, exposed to CPC rinses, as measured by optical density at six passages. The products are ranked from left to right in order of increasing optical density at six passages with saliva. For the same product, with one exception (Search), saliva increased the staining and this effect was highly significant ($P < 0.001$). Overall the CPC rinses varied considerably and highly significantly ($P < 0.001$ parametric and non-parametric ANOVA) in staining despite, for most, containing the same concentration of CPC. In particular, the product Reach (Rch) produced staining that was little different from water (W). All other CPC rinses produced more staining than water.

Fig. 2 shows the staining by the non-CPC products at six passages with and without saliva. Again saliva variably increased staining for most products. The products Toms (Tom), Actibrush (Act) and Mentadent (Ment) were little different to water. Corsodyl (Cors), the chlorhexidine product, and Oraldene (Oral) produced the expected staining; for Oraldene this occurred particularly in the presence of saliva. Listerine similarly produced considerable staining in the presence of saliva.

Experiment 2

Fig. 3 shows the tea staining induced by CPC rinses on saliva and no-saliva treated blocks as measured by optical density at six passages. The same pattern for the effects of saliva and the different solutions noted in Experiment 1 are apparent. Saliva significantly increased staining for all CPC solutions including the previous exception, Search ($P < 0.001$), and treatments differed highly significantly overall ($P < 0.001$ parametric and non-parametric ANOVA). Reach was again little different from water. Without saliva Search (Sch) and Sainsbury’s Oral Health (SOH) produced similar staining to the stock 0.05% and 0.1% CPC solutions. However the same two products induced considerably less staining than the stock solutions in the presence of saliva.

Experiment 3

This compared the chlorhexidine mouthrinse products Corsodyl (0.2%) and Eludril (0.1%).

Fig. 4 shows the tea staining of blocks exposed to neat solutions of the two chlorhexidine products and the manufacturer’s recommended 1 in 3 dilution of the Eludril product. At 12 passages there was overall a small but statistically significant difference ($P < 0.01$) in optical density readings. This arose from significantly more staining with the two Eludril solutions compared with the Corsodyl solution ($P < 0.05$-0.01).
DISCUSSION

Polymethylmethacrylate has been used in a number of experiments *in vitro* to study the adsorption and staining potential of cationic antiseptics, particularly for chlorhexidine and CPC. The model has clinical relevance since acrylic is a commonly used restorative dental material and staining of these materials *in vivo* is a well-known consequence of the use of cationic antiseptic mouthrinses. Moreover, the results for dietary staining of acrylic by certain antiseptics complement the findings for enamel both *in vitro* and *in vivo*.

In the first instance, the results of this study confirm several investigations that chlorhexidine and CPC can bind dietary chromogens and food dyes to surfaces *in vitro* and teeth and mucosal surfaces *in vivo*. Moreover, staining progressively increased with increasing frequency of exposure of a surface to the cation and then the chromogen. Saliva pretreatment of specimens, notably for CPC, increased staining as shown by the optical density readings at the equivalent number of passages. Why saliva increases staining, particularly for CPC, may result from increased adsorption of this antiseptic due to the availability of proteinaceous material for which cationic antiseptics have an affinity. This may explain the considerable increase in staining by Oral B and Listerine in the presence of saliva and suggests limited adsorption of these products to naked acrylic. An increased uptake of tea chromogens into the pellicle layer is unlikely as saliva treatment of water controls had little influence on staining.

Staining by the chlorhexidine rinse and some CPC rinses was essentially similar and several factors have to be considered in explaining this. First, the concentration of the chlorhexidine rinse was twice to four times that of the CPC rinses (0.2% vs 0.10% or 0.05%). Although the available dose of both antiseptics was almost certainly sufficient to saturate all receptor sites. Secondly, adsorption isotherms indicate that considerably more CPC than chlorhexidine is adsorbed to acrylic. However, CPC is monocationic and chlorhexidine dicationic and differences in interaction potential once adsorbed may explain why CPC does not stain more than chlorhexidine. Indeed, the critical concentration for the precipitation of tea by CPC *in vitro* is higher than chlorhexidine.

The second observation in Study 1 was that one other product, Listerine, produced staining both without and, more particularly, with saliva. The possibility of staining associated with Listerine was alluded to by Mandel.

Since this time, at least one study has demonstrated significant staining clinically, albeit less than chlorhexidine, when Listerine was used in the absence of normal toothbrushing. Indeed, this may be an important factor in extrapolating this *in vitro* data to the clinical situation. Certainly, the methodology appears to accurately predict the potential of agents to stain *in vivo*. However, it cannot assess how readily the stain can be removed by toothbrushing: as such this factor may account for the failure to observe that Listerine can cause extrinsic toothstaining. Moreover, over the counter CPC and essential oil/phenolic rinses are not typically used by the general public without toothbrushing nor are they normally supervised by a professional. Chlorhexidine on the other hand has been used in many studies and clinical situations where mechanical toothcleaning is limited or temporarily halted. Additionally, professional supervision is common. Both factors would tend to encourage the occurrence and recognition of staining.

Whilst this methodology could predict differences in clinical staining for products having a common ingredient, it cannot predict differences in staining for products with different active ingredients. The major reason for this limitation is the oral substantivity of particular agents. Staining *in vitro* by CPC and some CPC products was similar to the chlorhexidine rinse, however the substantivity *in vivo* of CPC is much less than that of chlorhexidine. Thus, given a twice daily rinsing regimen, the time available for chlorhexidine to interact with dietary chromogens is far greater than that for CPC. In this respect increasing the frequency of CPC rinses enhances plaque inhibition and it could be predicted that this would increase staining.

Other than the confirmatory nature of the observation for tea and CPC or chlorhexidine interactions, the findings are more important for two reasons. This study clearly demonstrates that some variation in staining occurs with different CPC products containing apparently similar concentrations of the antiseptic. Most products, for which information was available or obtained, contained 0.05% CPC, although some were 0.1% CPC. The standard solutions indicated, however, that staining differs little over a 0.05-0.1% concentration range. Interestingly, the Sainsbury Extra Strength rinse (SES) contains 0.3% CPC and this product stained more than any other CPC product. Listerine and Listerine with fluoride both with and without saliva showed considerably less staining than most other CPC products. This would suggest the availability of the CPC in these products was less than the incorporated 0.05%. Unfortunately, there are no clinical data to determine the significance of this potential availability difference to plaque control. More noticeable was the almost total lack of potential to cause staining with the Reach product. By comparison with a standard CPC solution or the water controls it must be concluded that the CPC is unavailable in this product. In this case, the results would be consistent with an independent study demonstrating the Reach product produced no plaque inhibition greater than saline in a 4-day plaque regrowth trial. Other variations between the various CPC products may also be explained by other ingredients, as with Reach, reducing CPC activity or conversely increasing CPC activity. Unfortunately, exact formulae details are difficult to obtain from manufacturers and an explanation for such differences must remain subject to speculation.
In conclusion, this method in vitro appears a useful and simple technique to predict the potential of over-the-counter and prescription mouthrinses to cause extrinsic staining of the teeth. As important, for formulations containing the same cationic antiseptic the method will detect the comparative availability of the agent, and certainly could help to predict the clinical efficacy of a product. Thus, with a previous chlorhexidine product, this study would tend to explain the poor performance of a CPC product to be because of the unavailability of the active ingredient.

References

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