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A methodology using subjective and objective measures to compare plaque inhibition by toothpastes

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Abstract

Objectives: Plaque scoring usually employs subjective indices. The aim was to compare plaque inhibition of three toothpastes using two objective and one subjective measures of plaque.

Material and Methods: Formulations were: (1) an experimental anti-plaque paste (test); (2) an experimental paste (minus active or negative control); and (3) a proprietary anti-plaque toothpaste product (positive control). The study was a blind, randomised crossover design using a 4-day, no tooth brushing, plaque regrowth model and involving 22 healthy subjects. After baseline plaque removal, subjects rinsed twice a day with slurries of the allocated paste. On day 5, plaque was scored by index, wet weight and optical density of extracted disclosing solution from the plaque (stain intensity).

Results: All data showed the same pattern. There were highly significant subject and treatment effects but not period effects. The positive control was highly significantly more effective in plaque control than the test and minus active experimental formulations, which in turn were not significantly different from each other. There were strong and significant correlations between pairs of scoring methods particularly wet weight and stain intensity.

Conclusion: The use of objective methods of plaque alongside conventional subjective indices provided convincing evidence for increased discriminatory power in a study comparing plaque inhibition by toothpastes.

Key words: clinical trial; plaque inhibition; plaque measurement; plaque

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Oral-care devices, formulations and products have been chronicled for at least 6000 years (for reviews see Fischman 1992, 1997). The list includes an array of agents and materials, and some recipes for mouthrinses, toothpastes and toothpowders seem, by today's standards, quite bizarre, if not disgusting. The scientific evaluation of oral-care products is quite a new phenomenon with perhaps the first randomised double-blind controlled crossover study in dentistry conducted as recently as 1960 by Cooke & Armitage (for review see Addy & Moran 1997). The marketing of the nylon filament plastic handled manual toothbrush in the 1920s must be seen as one of the major achievements in the 6000-year history of oral-care products (Fischman 1997). Similarly important were the findings of the association and then aetiological role of plaque with gingivitis (Ash et al. 1964, Loe et al. 1965). Unfortunately, both discoveries led to the rather simplistic view that if everyone regularly cleaned their teeth with a toothbrush, there would be no gingivitis and thereby no periodontal disease. The recognition that both compliance and dexterity with tooth cleaning habits was of critical importance to outcome quickly followed (for review see Frandsen 1986) and in part presumably led to the search for chemical agents, which might be adjunctive to mechanical plaque control. Indeed, the early literature on chlorhexidine mouthrinses might be interpreted as an attempt to

find an alternative to mechanical cleaning (Loe & Schiott 1970).

Reviews on the subject of adjunctive chemical plaque control have tended to conclude that the toothpaste vehicle is the most appropriate, if only because of the cost/benefit ratio (Addy et al. 1992, Addy & Moran 1997) and the use of toothpaste on toothbrushes as the most common form of oral hygiene (Frandsen 1986). Despite extensive research and numerous clinical trials, the number of chemicals that have been successfully formulated into toothpastes to provide adjunctive plaque control with little or no side effects, albeit usually cosmetic, have been few (for reviews see Cummins 1997, Moran 1997, Eley 1999). Moreover, so far, no chemical agent can be considered good or successful for periodontal disease, as fluoride in toothpaste is for caries.

The evaluation of such oral hygiene products is usually a step-by-step process (for reviews see Addy 1995, Addy & Moran 1997) and in the early phases usually involves the testing for chemical effects alone against plaque regrowth. Such studies rely for the most part on subjectively scored indices, which in turn rely on the judgement of a clinical examiner (for review see Fischman 1986). Attempts have been made to introduce objectivity into plaque scoring some of which date back many years, including planimetry from photographs (Arnim 1963), digital imaging (Eaton et al. 1985) and plaque weight (Marthaler et al. 1961).

The aim of the present study was to compare plaque inhibition by three toothpastes using two objective and one commonly used subjective measures of plaque. The study was first and foremost methodological and not for product claim support, therefore, reference to the formulations will only be descriptive.

Material and Methods

The study involved healthy volunteers and approval was obtained from the United Bristol Healthcare Trust Ethics Committee. The design, conduct, monitoring, analysis and reporting of the study was in accordance with the Guidelines for Good Clinical Practice. Subjects were recruited into the study after they had received verbal and written information concerning the study, given signed consent to participate and satisfied the inclusion criteria. The investigation used the 4-day plaque regrowth model developed to study the chemical plaque inhibitory properties of toothpaste (Addy et al. 1983). The study was a single, examiner blind, randomised, three-treatment crossover design, in which up to 25 subjects of either gender were to be recruited. Subjects had to be over 18 years of age, dentate without removable dental prostheses or fixed or removable orthodontic appliances, with a high standard of oral hygiene and gingival health, and at least 16 teeth deemed scorable on the buccal surfaces. A medical history was obtained from each subject to exclude those on pharmacotherapy or with medical conditions, which might compromise the conduct of the study. Prior to commencing the study, subjects were provided with proprietary fluoride toothpaste to use for at least 3 days before the first period of the study and during the 9-day washout periods between the first and second and second and third periods. The toothpaste formulations under test were:

1. a sodium lauryl sulphate detergent-based, experimental ''anti-plaque'' dentifrice formulation (test);
2. a sodium lauryl sulphate detergent-based, experimental dentifrice formulation (minus active or negative control); and
3. a sodium lauryl sulphate detergent-based proprietary ''anti-plaque'' toothpaste product (positive control).

The toothpastes were used as slurries with 3 g dispersed in 10 ml of water. Rinsing was for 60 s and supervised. At the commencement of each study period, subjects were rendered plaque free by a professional prophylaxis and they suspended normal tooth cleaning and used the allocated slurry. Rinsing was once on the afternoon of day 1, twice, morning and afternoon of days 2, 3 and 4, and once on the morning of day 5. On the afternoon of day 5, the buccal surfaces of all teeth were disclosed with an erythrosine dye and each tooth was scored according to the criteria of the Turesky et al. (1970) modification of the Quigley & Hein (1962) plaque index. All scoring was by a single examiner. A second clinician then removed all of the visible buccal plaque into pre-weighed 2 ml capped glass vials and the vials reweighed to obtain the plaque wet weight. A 0.2 ml volume of water was then added to each vial containing the plaque samples and mixing carried out using a Whirlimixer (Fisons Scientific Equipment, Loughborough, Leicester, UK). The plaque samples in the sealed vials were then sent to the laboratory where the erythrosine was extracted from the samples by adding 1 ml of ethanol to each vial and letting stand at 37°C for at least 18 h. Samples were then centrifuged and the stain intensity in the supernatant was determined by measuring the optical density of the extract on an UV/visible spectrophotometer at a wavelength of 532 nm. At screening and baseline and scoring visits for each period, the plaque examiner conducted an oral soft-tissue examination and also a verbal enquiry concerning any adverse events at the scoring visits.

Statistical analysis

The rationale for the sample size was based on the power of numerous previous studies using the present protocol. Additionally, since the study by virtue of the plaque collection was explanatory in nature, the upper limit of 25 subjects was set by the limitations of the logistics of the design. Total mouth mean buccal plaque index, wet plaque weight and stain intensity readings were the outcome measures. Based on a normal distribution of the data, analysis of variance was performed modelled on the effects of subject, period and treatment. Paired comparisons between treatments were made using paired *t*-tests. Pearsson's correlation coefficients were then calculated between the three measures of plaque accumulation.

Results

A total of 22 subjects (12 female, 10 male, age range 20–30 years) were recruited and commenced the study. One subject did not complete one period (negative control treatment) and data were not available for one subject for plaque wet weight (negative control treatment). No adverse events were reported or soft-tissue pathology noted. The means and standard errors for mean buccal plaque index for each treatment are shown in Table 1, together with the same for plaque weight and plaque extract. Observationally, all of the data show the same pattern, with the lowest scores found for the positive control, with essentially similar scores for the

Table 1. The mean (standard error) plaque index, plaque weight and plaque extracted for the three toothpastes after 4 days of no oral hygiene

	Test	Positive control	Negative control
plaque index	3.027 (0.072)	2.359 (0.072)	3.167 (0.075)
plaque weight	0.850 (0.080)	0.271 (0.080)	0.959 (0.083)
plaque extract	11.56 (0.99)	3.014 (1.00)	12.18 (1.08)

test and negative control formulations. Proportionately, the magnitude of the differences between the positive control and the test and negative control formulations can be more easily appraised from the plaque wet weight and stain intensity data.

Analysis of variance revealed highly significant subject and treatment differences ($p<0.0001$), but no evidence of significant period effects ($p>0.05$). Paired comparisons for all three measurements revealed highly significant differences in favour of greater treatment effects for the positive control compared with the test and negative control formulations ($p<0.002$–<0.0001). There were no significant differences between the test and negative control formulations for any measurement parameter. Correlation coefficients between index and weight and index and stain intensity were 0.64 and 0.58, respectively, and between plaque weight and intensity 0.82: all correlations were highly significant ($p<0.0001$).

Discussion

The present study had a single aim, namely to determine whether objective measures of plaque could be used alongside a more conventional subjective plaque index to compare the plaque inhibitory properties of toothpastes. Intentionally, the formulations are referred to in descriptive terms, as the study specifically was not performed to generate data for product claim support, particularly since the test and negative control were experimental formulations. The only expectation, consistent with the methodology, was that the positive control would be more active than the negative control. The aim appears to have been achieved. Thus, the lack of differences and the differences, respectively, in plaque accumulation between the formulations could be shown by both the subjective measure, plaque index and the more objective measures, plaque weight and stain intensity. Indeed, the expected differences in favour of the positive control over the negative control were proportionately much more apparent with the objective measures. On reflection, this should have been expected if the objective measures were truly objective. Plaque weight and stain intensity are quantitative continuous scales whereas most plaque indices, including the Turesky et al. (1970) index, are ordinal. This difference in the measurement methods would be expected to result in greater discriminatory power with quantitative continuous scales, as was the case in the present study. The greater and similar discriminatory power of the two objective measures over the subjective method is indirectly supported by the correlation coefficients. Although all three paired correlations were statistically highly significant, the coefficients were much stronger between the two objective measures than between the objective measures and the subjective measure. Discriminatory power of plaque indices has been the subject of little research. Quirynen et al. (1991) compared five indices in a single study and, interestingly as here, found the objective method, which was planimetric area from photographs, the best. In apparent contradiction, Addy et al. (1999) using data from 15 studies reported greater discriminatory power with plaque index over planimetric plaque area in 10 of the studies. It must be remembered, however, that the plaque area method used (Addy et al. 1983) in the 15 studies reanalysed (Addy et al. 1999) has a subjective element unlike the photographic technique (Quirynen et al. 1991). One potential drawback of most plaque measurement techniques including objective planimetric techniques is the two-dimensional nature of recording. Both plaque weight and stain intensity relate to the three-dimensional nature of plaque. By virtue of this, they could provide valuable information as to the association of plaque mass to gingivitis and to the individual variability in the development of gingivitis as plaque accumulates. Additionally, such measures may have greater discriminatory power for gingivitis studies; however, all these hypotheses would need testing in randomised clinical trial protocols.

Ideally, outcome measures for any clinical trial of treatment should be chosen to provide the greatest discriminatory power, although this may have to be balanced against the logistics of applying the measure in the clinical setting or the degree of difficulty, time and cost of deriving the measure. In the present study, time for each measure was not recorded, but plaque collection and weighing were clearly more time consuming and could not easily have been performed without a second examiner. Stain extraction and intensity measurements are laboratory procedures, relatively straightforward and not dependent upon subject availability.

In conclusion, the use of more objective measures of plaque alongside a conventional subjective index provided convincing evidence for increased discriminatory power in studies comparing plaque inhibition by toothpastes. Such methods could find use in other protocols evaluating oral hygiene products.

References

Addy, M. (1995) Evaluation of clinical trials of agents and procedures to prevent caries and periodontal disease: choosing products and recommending procedures. *International Dental Journal* **45**, 185–196.

Addy, M. & Moran, J. M. (1997) Evaluation of oral hygiene products: science is true; don't be misled by the facts. In: *Toothpaste, Mouthrinse and other Topical Remedies in Periodontics*, eds. Addy, M. & Moran, J. M. *Periodontology 2000* **15**, 40–51.

Addy, M., Renton-Harper, P. & Newcombe, R. G. (1999) Plaque regrowth studies: discriminatory power of plaque index compared to plaque area. *Journal of Clinical Periodontology* **26**, 110–112.

Addy, M., Slayne, M. A. & Wade, W. G. (1992) The formation and control of dental plaque – an overview. *Journal of Applied Bacteriology* **73**, 269–278.

Addy, M., Willis, L. & Moran, J. (1983) The effect of toothpaste and chlorhexidine rinses on plaque accumulation during a 4 day period. *Journal of Clinical Periodontology* **10**, 89–98.

Arnim, S. S. (1963) The use of disclosing agents for measuring tooth cleanliness. *Journal of Periodontology* **34**, 227–245.

Ash, M. M. Jr., Gitlin, B. N. & Smith, W. A. (1964) Correlation between plaque and gingivitis. *Journal of Periodontology* **35**, 424–429.

Cooke, B. E. D. & Armitage, P. (1960) Recurrent Mikuliczs aphthae treated with topical hydrocortisone hemisuccinate sodium. *British Medical Journal* **1**, 764–766.

Cummins, D. (1997) Vehicles: how to deliver the goods. In: *Toothpaste, Mouthrinse and other Topical Remedies in Periodontics*, eds. Addy, M. & Moran, J. M. *Periodontology 2000* **15**, 84–99.

Eaton, K. A., Kieser, J. B. & Baker, R. (1985) Assessment of plaque by image analysis. *Journal of Clinical Periodontology* **12**, 135–140.

Eley, B. (1999) Antibacterial agents in the control of supragingival plaque – a review. *British Dental Journal* **27**, 286–296.

Fischman, S. L. (1986) Current status of indices of plaque. *Journal of Clinical Periodontology* **13**, 371–374.

Fischman, S. L. (1992) Hare's teeth to fluorides, historical aspects of dentifrice use. In: *Clinical and Biological Aspects of Dentifrices*, eds. Embery, G. & Rolla, G., pp. 1–7. Oxford: Oxford United Press.

Fischman, S. (1997) Oral hygiene products: how far have we come in 6000 years. *Periodontology 2000* **15**, 7–14.

Frandsen, A. (1986) Mechanical oral hygiene practices. In: *Dental Plaque Control Measures and Oral Hygiene Practices*, eds. Loe, H. & Kleinman, D. V., pp. 93–116. Oxford: IRL Press.

Loe, H. & Schiott, C. R. (1970) The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *Journal of Periodontal Research* **5**, 79–83.

Loe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 177–187.

Marthaler, T., Schroeder, H. & Muhleman, H. (1961) A method for the qualitative assessment of plaque and calculus formation. *Helvetica Odontologica Acta* **5**, 39–45.

Moran, J. M. (1997) Chemical plaque control-prevention for the masses. In: *Toothpaste, Mouthrinse and other Topical Remedies in Periodontics*, eds. Addy, M. & Moran, J. M. *Periodontology 2000* **15**, 109–117.

Quigley, G. & Hein, J. (1962) Comparative cleansing efficiency of manual and power brushing. *Journal of the American Dental Association* **65**, 26–29.

Quirynen, M., Dekeyser, C. & Van Steenberge, D. (1991) Discriminatory power of five plaque indices. *Journal of Periodontology* **62**, 100–105.

Turesky, S., Gilmore, N. D. & Glickman, I. (1970) Reduced plaque formation by the chloromethyl analogue of Victamine C. *Journal of Periodontology* **41**, 41–43.

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