J Clin Periodontol 2005; 32: 545–548 doi: 10.1111/j.1600-051X.2005.00709.x

Journal of Clinical Periodontology

The prevention of plaque re-growth by toothpastes and solutions containing block copolymers with and without polypeptide

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Claydon NC, Addy M, Newcombe R, Moran J. The prevention of plaque re-growth by toothpastes and solutions containing block copolymers with and without polypeptide. J Clin Periodontol 2005; 32: 545–548. doi: 10.1111/j.1600-051X.2005.00709.x. © Blackwell Munksgaard, 2005.

Abstract

Background/Aims: Chemicals which have a direct effect at inhibiting or reducing bacterial adherence to tooth surfaces may subsequently inhibit plaque growth and reduce gingival inflammation. This study investigated whether two anti-adherent systems could inhibit plaque re-growth in the mouth when rinsed as a solution or as a toothpaste slurry.

Methods: A total of 21 subjects took part in a partially blind, seven cell cross-over study which compared the effects on plaque re-growth of a binary system containing block copolymers, a ternary system containing block copolymers and polypeptide, both used as toothpaste slurry rinses, their corresponding solution rinses, a conventional fluoride toothpaste rinse, a positive control chlorhexidine rinse and a negative water control. Following a dental prophylaxis subjects then rinsed with 10 ml of one of the test products for 1 min. twice a day over a 4-day period. Throughout each trial period the subjects were not permitted to use any other forms of oral hygiene. On the fifth day (96 h), the volunteers returned to the clinic, and plaque was assessed by (1) plaque index and (2) plaque area following disclosing with a food dye. The test phase of the trial was repeated for each agent and was followed by a 10-day ''washout'' period.

Results: Essentially neither of the anti-adherent systems inhibited plaque re-growth, whether administered in a toothpaste slurry or solution compared with the controls. If anything, neither of the test pastes were as effective as the marketed commercial paste ($p < 0.001$). As expected plaque recorded following use of the chlorhexidine rinse was significantly less than that seen with any of the other rinses ($p < 0.001$).

Conclusions: Using this 4-day plaque re-growth model, the findings of this study failed to show any benefit in using the anti-adherent systems, either in a rinse or toothpaste, with the aim of inhibiting or reducing plaque formation.

Key words: anti-adherents; clinical trials; plaque; toothpastes

Accepted for publication 28 September 2004

Dental plaque is considered to be the key factor associated with both dental caries and gingival inflammation. The latter, if untreated may extend beyond the gingival margin and progress to periodontitis. This may ultimately lead to the loss of teeth. Approaches to control dental plaque centre on mechanical removal by tooth cleaning or by the use of chemicals, which prevent or reduce bacterial multiplication (Addy 1986). An alternative approach to control plaque is to inhibit or reduce bacterial adherence to the tooth surface, which theoretically would result in less plaque and conceivably less gingival inflammation. Such an approach is not new, with one such system based on a high mole-

cular weight copolymer (M239144) initially showing promise at reducing bacterial adherence to hydroxyapatite in vitro (Slayne et al. 1994). However, subsequent studies failed to show a direct antibacterial effect in vitro (Wade et al. 1994) and a reduction in plaque in vivo (Moran et al. 1995, Claydon et al. 1996) More recently systems using block copolymer polyoxypropylene/polyoxyethlylene (PO/EO), silicones or casein derived peptides have been tested in vitro to determine their effects on adherence of *Streptococcus sanguis* to hydroxyapatite coated surfaces (Guan et al. 2001). The findings of this study confirmed the anti-adherence properties of the copolymer and peptides, with some reduction in activity noted when incorporated into a toothpaste. Similarly, binary PO/EO block copolymer systems and ternary PO/EO systems with peptide have been investigated using the same in vitro methodology (Guan et al. 2003a, b). Synergistic effects at reducing bacterial adherence were observed by both binary combinations of copolymer (Guan et al. 2003b) and ternary combinations of copolymer and peptide (Guan et al. 2003a). The aim of the present study was to determine the plaque inhibitory capacity of two new novel anti-adherent systems applied both in an aqueous solution and in a toothpaste slurry. For comparative purposes a currently marketed fluoride toothpaste was evaluated together with a negative water control and a positive chlorhexidine mouthrinse control.

Materials and Methods

Plaque inhibitory efficacy alone was measured in this study. A single centre, single (examiner) blinded, seven period, seven treatment, randomized cross-over design was used for this study. This design consists of three 7 by 7 Latin squares, so that each treatment was used by exactly three volunteers in each period. Using this design would result in balance for any carry-over effect. The two test toothpastes contained either a binary block (PO/EO) copolymer (binary paste) or a binary block (PO/EO) copolymer with polypeptide (ternary paste). Solution rinses contained the equivalent systems at the same concentration. The benchmark control paste (Addy 1986) was a commercial fluoride product (Boots Regular Fluoride, Boots Company, Nottingham, UK), which contained none of the aforementioned ingredients. The other control rinses contained 0.2% chlorhexidine and sterile water. The study was conducted in accordance with the Declaration of Helsinki (1964) and subsequent amendments. Prior to the start of the study, ethical approval was obtained from the University of Bristol Health Care Trust and volunteers provided signed and witnessed consent to participate. The study was designed, conducted, analysed and reported according to guidelines for Good Clinical Practice.

A total of 21 volunteers took part [comprised nine males and 12 females ages ranging from 22 to 64 years (mean = 30.0 years)]. The volunteers were dentate with a catalogued high standard of oral hygiene and gingival health. Volunteers who wore fixed or removable appliances or dental prostheses were excluded, as were those with any medical or pharmacological history that could compromise the conduct of the study. The subjects were given a standard fluoride dentifrice (Boots Regular Fluoride, Boots Company) and toothbrush (Boots Adult, Boots Company) to use in place of their normal products 1 week before the study and during each washout phase. Because of the potential carry-over effects of the chlorhexidine rinse (Newcombe et al. 1995), washout periods were of 10 days duration. On day one (Monday) of each trial period, the subjects had their plaque disclosed with Plaque Finder 2 tone–Blue #1 and FD & C Red #38 (Oraldent Ltd, Kimbolton, UK) and were then given a dental prophylaxis to clean their teeth free of plaque and calculus deposits. After this, they were instructed to rinse with the allocated slurry or solution rinse thoroughly for 1 min. For the toothpaste slurries, these were prepared by dissolving 3 g of toothpaste in 10 ml of distilled water. The subjects were instructed to return to the clinic that afternoon and told again to rinse their mouths with 10 ml of the allocated preparation for 1 min. For the following 3 days at the same times, in the morning and afternoon, they repeated the rinsing procedure. During this period the subjects were instructed not to use any other forms of oral hygiene, i.e. toothbrushing, flossing, etc. Ninety-six hours after the initial prophylaxis (Friday) the volunteers returned to the clinic when their teeth were disclosed with the aforementioned dye. The area of plaque on the buccal surfaces of the upper and lower incisors, canines and premolars was drawn onto standard tooth charts according to the method described by Addy et al. (1983). The area of plaque on each tooth was then measured with a Cherry digitiser (Wacom Europe GmbH, Krefeld, Germany) in conjunction with a microcomputer and DCAD software package.

In addition, plaque on the buccal and lingual surfaces of all teeth except third molars was assessed using the Turesky et al. (1970) modification of the Quigley & Hein (1962) plaque index. During the following 10 days the subjects used the toothbrush and washout dentifrice provided at home, in place of their normal toothbrush and dentifrice.

Statistical analyses

Data were included on all of the subjects of the study. The primary analysis compared the whole mouth mean plaque score and plaque area values.

ANOVA was used to model the primary outcome variables on three factors, subject, period and treatment. Point and interval estimates were calculated for predetermined contrasts between chosen treatments. In addition subsets of plaque data at specific areas of the dentition were determined but were not analysed using the pre-determined contrasts.

Results

No untoward side effects were noted for any of the subjects for any of the preparations used as assessed by (1) examination of both hard and soft tissues of the mouth (2) recording any adverse events experienced or reported by the subjects themselves. None of the subjects were either suspected or known to have seriously violated the protocol. Out of the 21 who started the study, 18 completed with an entire set of data. The three subjects concerned failed to attend on one of their assessment days because of social commitments. As such the data set was not quite orthogonal, so that the calculated mean plaque levels on each treatment required slight adjustment for possible confounding with subject or period differences. The distribution for plaque index and plaque area did not display consistent evidence of skewness and analyses based on the Gaussian distribution without transformation was deemed appropriate.

ANOVA showed a highly significant variation between subjects ($p < 0.001$)

at 96 h for both plaque score and area. There was a moderate, statistically significant difference between the seven periods ($p = 0.019$ Turesky, 0.013 area) while overall treatment differences were also highly significant ($p < 0.001$). The mean whole mouth plaque scores and plaque area on the buccal surfaces of the teeth at 96 h are shown in Table 1. Selected statistical comparisons between test and control rinses are shown in Table 2. As expected the least amount of plaque was seen with the chlorhexidine rinse. This was followed by the control paste with significantly reduced plaque compared with both binary and ternary pastes ($p < 0.001$). There was no evidence to show any difference in activity between the two test pastes themselves, their two solutions and water control ($p > 0.05$). This was true whether they were assessed by plaque area or plaque index.

Discussion

There are a limited number of clinical studies, which can be cited on the use of anti-adherent chemicals to control dental plaque (Moran et al. 1995, Claydon et al. 1996). Overall, these studies have also failed to demonstrate any advantage of the use of anti-adherents at least in mouthrinses. The decision to undertake a clinical study is very often based on preliminary laboratory studies which may predict possible clinical efficacy for a particular system. For the present study, in vitro studies had demonstrated some effects of anti-adherents on reducing bacterial attachment to surfaces, both for a binary PO/EO copolymer system (Guan et al. 2003b) and a ternary system with peptide (Guan et al. 2003a).

Of further interest was whether such systems behaved in a similar fashion in solution and when incorporated into another vehicle such as a toothpaste. With reference again to in vitro studies some reduction in activity has been noted when the anti-adherent system was incorporated into the toothpaste compared with the solution (Guan et al. 2001, 2003a). The results of the present study would, if this is the case, suggest poor correlation of in vitro effects with that seen clinically. Similar lack of correlation of laboratory and clinical studies have been previously seen (Slayne et al. 1994, Wade et al. 1994, Moran et al. 1995, Claydon et al. 1996). The poor performance of the binary and ternary blocking anti-adherents in toothpaste is all the more surprising because they were less effective than a benchmark toothpaste at reducing plaque. The reduced activity compared with the commercial paste may suggest formulation problems with the test pastes. It is noted that both the test pastes contained a surfactant, the nature of which was not stated. If the pastes did contain the same detergent as the commercial paste, such as sodium lauryl sulphate, some activity at least equivalent to the commercial paste would have been expected. Clearly, some negation of activity by the anti-adherents themselves on the toothpaste detergent could result in a significant reduction in activity. It is interesting to note that in vitro, an anti-adherent chemical reduced the antibacterial activity of chlorhexidine (Wade et al. 1994). Similarly in a subsequent clinical study a copolymer anti-adherent mouthrinse, in addition to being ineffective, was also shown to inactivate chlorhexidine's antiplaque activity (Claydon et al. 1996). It is also worthwhile to point out that the methodology used in the present study i.e. the 4-day plaque re-growth study, does not involve daily removal of any plaque which has formed. Once established it is possible that any beneficial effect by an anti-adherent system on plaque is lost and is only effective in preventing new deposits. Equally, it is possible that although quantitative effects are not seen, qualitative effects on the bacterial

Table 1. Plaque area and scores for all sites following use of rinses for 96 h

RX	Plaque area mean (SD)	Turesky Index mean (SD)
Binary paste*	1.09 (0.41)	2.30 (0.43)
Ternary paste*	1.08 (0.43)	2.71 (0.43)
Binary rinse*	1.26 (0.47)	2.84 (0.48)
Ternary rinse†	1.23 (0.49)	2.83 (0.50)
Control paste†	0.75 (0.42)	2.34 (0.48)
Water†	1.20 (0.47)	2.84 (0.44)
Chlorhexidine†	0.33 (0.27)	1.45 (0.50)

*$n = 20$; †$n = 21$;

ANOVA treatment differences = $p < 0.001$ for both plaque area and Turesky Index.

Table 2. Whole mouth mean plaque area and Turesky scores

Contrast	Whole mouth mean Turesky scores			Whole mouth mean plaque area		
	Adjusted difference	95% confidence interval	*p*-value	Adjusted difference	95% confidence interval	*p*-value
Binary paste* *versus* CHX†	+1.28	+1.10 to+1.45	<0.001	+0.76	+0.62 to 0.90	<0.001
Ternary paste* *versus* CHX†	+1.30	+1.13 to+1.47	<0.001	+0.80	+0.66 to 0.93	<0.001
Binary rinse* *versus* CHX†	+1.39	+1.22 to+1.56	<0.001	+0.92	+0.78 to+1.05	<0.001
Ternary rinse† *versus* CHX†	+1.39	+1.22 to+1.56	<0.001	+0.90	+0.77 to+1.03	<0.001
Binary paste* *versus* Ctrl paste†	+0.38	+0.21 to+0.55	<0.001	+0.34	+0.21 to+0.48	<0.001
Ternary paste* *versus* Ctrl paste†	+0.41	+0.24 to+0.58	<0.001	+0.38	+0.24 to+0.51	<0.001
Binary rinse* *versus* water†	− 0.005	− 0.18 to+0.17	0.96	+0.049	− 0.09 to+0.18	0.48
Ternary rinse† *versus* water†	− 0.006	− 0.18 to+0.16	0.94	+0.030	− 0.10 to+0.16	0.66
Binary* *versus* ternary paste*	− 0.023	− 0.20 to+0.15	0.79	− 0.037	− 0.17 to+0.10	0.59
Binary* *versus* ternary rinse†	+0.002	− 0.17 to+0.17	0.98	+0/019	− 0.12 to+0.15	0.78

Contrasts between selected pairs of treatments.

*$n = 20$; †$n = 21$;

CHX, Chlorhexidine; Ctrl, Control.

composition of plaque may be evident. Thus beneficial effects on gingivitis cannot be ruled out.

In conclusion, accepting the limitations of the 4-day plaque re-growth model, this study failed to show any value in using the present anti-adherent copolymers, either in a rinse or toothpaste, with the aim of inhibiting or reducing plaque formation.

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