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Effect of triclosan/copolymer-containing toothpaste on the association between plaque and gingival bleeding: a randomized controlled clinical trial

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Abstract

Aim: To study longitudinal associations between plaque and gingival bleeding and multilevel variance/covariance structures after introducing triclosan-containing toothpaste.

Material and Methods: A 10-week, randomized, two-arm, double-masked, controlled clinical trial was conducted in 34 healthy, non-smoking females with plaque-induced gingivitis. Clinical periodontal examinations were repeated every other week. At week 4, test toothpaste containing 0.24% sodium monofluorophosphate, 0.3% triclosan, and 2% polyvinyl–methyl ether maleic acid; or control toothpaste containing 0.76% sodium monofluorophosphate and 0.1% sodium fluoride, were randomly distributed.

Results: Multivariate multilevel models indicated that, after introducing experimental toothpastes, subject random error was reduced from 0.6 to below 0.2. The odds ratio (OR) of bleeding on probing (BOP) was about 30% less in the test than in the control group ($p<0.01$). At the end of the experiment, ORs for BOP and plaque index scores 1–3 (reference 0) were 2.1–2.4 in the control group, but 1.1–1.9 in the test group ($p<0.05$). No effects on plaque levels and calculus were observed.

Conclusions: Multivariate multilevel modelling allows the study of fixed and random effects of experimental toothpastes on gingival inflammation in small sample. Triclosan appears to attenuate the causal association between supragingival plaque and gingival bleeding in gingivitis.

Key words: bleeding on probing; multilevel modelling; plaque; randomized-controlled trial; toothpaste; triclosan

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Although most cases of gingival disease are considered as entirely microbial plaque-induced (Mariotti 1999), intrinsic and extrinsic factors modulate the inflammatory response to dental plaque (Caton et al. 1999). Great individual differences in the inflammatory response to dental plaque have been described in gingivitis experiments (Lie et al. 1995, Fransson et al. 1996, Trombelli et al. 2004) and a steady-state plaque environment (Müller et al. 2000, Müller & Heinecke 2002). Factors which might dampen or enhance the local inflammatory response to dental plaque include pregnancy-associated (Raber-Durlacher et al. 1994) or menstrual-cycle (Machtei et al. 2004) hormonal fluctuations, stress (Deinzer et al. 2000), systemic non-steroidal anti-inflammatory drugs (Heasman et al. 1993, Royzman et al. 2004), or smoking (Danielsen et al. 1990, Lie et al. 1998, Müller et al. 2002).

As a compound of toothpastes and mouthwashes, triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether), a non-ionic antibacterial/antimicrobial agent incorporated in soaps and cosmetics, has been shown to dampen, the inflammatory response during experimental gingivitis. The anti-inflammatory action might be independent of its plaque-reducing effect (Stephen et al. 1990, Lindhe et al. 1993, Ramberg et al. 1995, Rølla et al. 1997), but results of recent gingivitis experiments are inconclusive (McClanahan & Bartizek 2002). Triclosan has the ability

to inhibit both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism with similar efficacy (Gaffar et al. 1995). In cell culture experiments, it was found that triclosan inhibited interleukin-1β-induced prostaglandin E_2 production by human gingival fibroblasts in a concentration-dependent manner and at relatively low concentrations (Modeer et al. 1996). In a recent meta-analysis of studies comparing plaque and gingivitis levels in adults using either triclosan/polyvinyl-methyl ether maleic acid-, or only fluoride-containing toothpaste with at least 6 months duration (Davies et al. 2004), triclosan-containing toothpaste was more effective regarding reduction of both plaque and gingivitis levels. Thus, it remains unclear whether the *association* between plaque and gingivitis is attenuated when triclosan-containing toothpaste is introduced. The aim of the present randomized-controlled clinical study was to determine effects of triclosan-containing toothpaste on longitudinal associations between the amount of supragingival plaque and gingival bleeding tendency in a steady-state plaque environment. Multivariate multilevel modelling was applied to explore site-specific associations and variance/covariance structures at the subject, tooth, and site levels over time. As it has been shown that smoking may modify the association under investigation (Müller et al. 2002), and gender seems to influence the periodontal response to plaque and poor oral hygiene (Waschul et al. 2003), the present study was conducted in female non-smoking adults with plaque-induced gingivitis.

Material and Methods

The study was a 10-week, randomized, two-arm, double-masked, controlled clinical trial. Its design had been reviewed and approved by Kuwait University Faculty of Dentistry's Ethical Committee as conforming to Ethical Principles for Medical Research Involving Human Subjects according to the World Medical Association Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm).

Volunteers

Participants were recruited among fifth and sixth year dental students and dental hygienists of Kuwait University Dental Center. Inclusion criteria were (i) female gender, (ii) mild plaque-induced gingival disease as defined as slight gingival oedema and redness in certain areas, and (iii) minimum amounts of supragingival calculus (CLS). The following exclusion criteria applied: (i) any indication for antibiotic prophylaxis; (ii) pregnancy or lactation as revealed by the volunteer; (iii) any long-term medication with a possible effect on gingival inflammation; (iv) any non-plaque-induced gingival disease; (v) destructive periodontal disease with a possible exception of localized gingival recession; (vi) extensive tooth restoration or tooth replacement; (vii) smoking. Based on sample size calculations, 34 eligible volunteers were invited to participate. They were between 19 and 28 years of age (mean standard deviation age 22.2 ± 1.4 years) and systemically healthy. The participants had a minimum of 23 erupted teeth (mean 28.7 ± 2.7), and, with a few exceptions (first molars due to caries), teeth had been extracted for orthodontic reasons. After written and oral briefing on the study's objectives, procedures, risks and benefits, volunteers gave their written consent for participation.

Experimental design

Figure 1 illustrates the outline of the study. Simple randomization was accomplished by assigning computer generated random numbers to volunteers by an independent periodontist (E. K.). Allocation to test and control groups was concealed to the principal author (H. P. M.) until the statistical analysis was completed. After randomization a prophylaxis session was provided 1 week before the commencement of the clinical trial. While toothpaste, toothbrushing frequency, and use of dental floss were recorded in a questionnaire, no attempt was made to improve the oral hygiene of the volunteers. Instead, they were advised not to alter their oral hygiene habits. However, in case a volunteer used already triclosan-containing toothpaste or mouthwash she was asked to change the product to a brand not containing triclosan. The first three examinations, which were conducted every other week, were considered the *preparatory phase* of the study during which individual, site-specific associations between plaque levels and bleeding on probing (BOP), and a possible Hawthorne effect were studied. It was assumed that potential effects of triclosan would be lost during these 4 weeks in those few volunteers who already used toothpastes containing the compound. Immediately after the 4-weeks' examination (a second Baseline), participants received neutral containers with, according to the randomization process, either *placebo* toothpaste (*control group*, $n = 17$): Colgate Maximum Cavity Protection, containing 0.76% sodium monofluorophosphate and 0.1% sodium fluoride (corresponding to 1450 p.p.m. F^-), but no triclosan; or *verum* toothpaste (*test group*, $n = 17$): Colgate Total (0.24% sodium monofluorophosphate, 0.3% triclosan, and 2% polyvinyl-methyl ether maleic acid (copolymer); both Colgate-Palmolive Company, Dammam, Saudi Arabia. Toothpastes were purchased over the counter. During the *experimental phase*, three further examinations took place every other week (Fig. 1).

Clinical examination

The principal investigator carried out all examinations. Clinical periodontal conditions were recorded at six sites of every tooth present. Periodontal probing depth (PPD) and clinical attachment level (CAL) were measured with a pressure-controlled probe (ClickProbe 1395, KerrHawe, Bioggio, Switzerland) to the nearest millimetre. The probe tip diameter is 0.5 mm and probing force, according to the manufacturer, 0.25 N. Thus, the approximate probing pressure is 1.27 MPa when the probe bends with a palpable click. After probing all facial sites of the first quadrant, gingival bleeding was assessed on a 0–2 scale (bleeding index, BI) where 1 was slight (single spot) and 2 profuse (the whole sulcus filled immediately with blood) BOP. Thereafter, facial sites of the

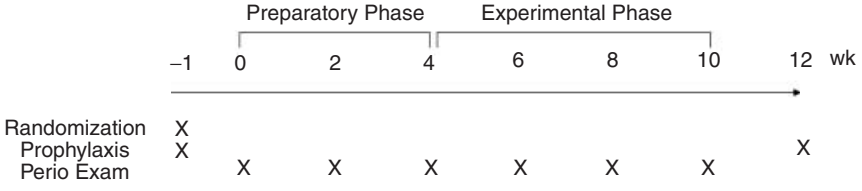

Fig. 1. Study design.

second quadrant were measured. Probing was continued at palatal sites, and in the mandible. Reliability of BOP after different time intervals had been assessed in a separate population (Müller & Barrieshi-Nusair 2005). Presence of CLS was recorded, and plaque disclosed with a plaque revelator (D&C Red-28, Sultan Chemists Inc., Englewood, NJ, USA). The amount of plaque was estimated using criteria of the plaque index (scores 0–3, PLI) system (Silness & Löe 1964).

Statistical analysis

The primary outcome variable was the association between plaque amount and BOP in a steady-state plaque environment. The null hypothesis was that triclosan-containing toothpaste does not change the association. For calculation of the minimum sample size, data of a comparable study in young adult smokers and non-smokers with plaque-induced gingivitis (Müller et al. 2002) were used for an estimate of the standard deviation of ln-transformed Mantel–Haenszel's common odds ratio (OR) for longitudinal associations, i.e. 0.5. A difference between test and control of 0.5 can then be detected at an α-level of 0.05 (two-sided) with a statistical power of 0.8 by enrolling 17 patients in each arm. An intention-to-treat approach was applied.

For secondary outcome variables (PPD, CAL, BI, BOP, PLI, CLS) data are given as mean ± standard deviation. Student's or paired *t*-tests were applied, as indicated. In a so-called ecological approach of data analysis (Müller et al. 2002) we first considered the correlation between mean plaque and BI before and after the introduction of experimental toothpastes, and calculated Pearson's *r*. We then used multilevel modelling of the binary response BOP (Yang et al. 2000, Goldstein et al. 2002). A four-level multivariate repeated measures binary response variance components model of BOP can be written as

$$y_{tjkl} \sim Bin(1,\ \pi_{tjkl}),$$

$$\log it(\pi_{tjkl}) = \sum_{t=1}^{n} \beta_{0,t} z_{tjkl} + \sum_{t=1}^{n} f_{tl} z_{tjkl} + \sum_{t=1}^{n} v_{tkl} z_{tjkl},$$

$$f_{tl} \sim N(0,\ \Omega_f),\ \ v_{tkl} \sim N(0,\ \Omega_v)$$

where n occasions are treated as repetition at level 1 (indicated by t) nested within sites (indicated by j), and teeth (indicated by k), nested in subjects l. Vectors of indicator variables (0, 1) for $t = 1, 2, \ldots, n$ are described by z_t. Residual terms at the subject and tooth levels associated with the intercept for each examination are designated as f_{tl} and v_{tkl}, respectively. The model can be extended by including time-dependent explanatory clinical variables in order to assess their respective associations with BOP at the respective occasion and any attenuation due to test toothpaste. There is no level 1 (occasion) variation, as at level 2 (site) binomial variates among occasions are allowed to covary within sites. At this level, a covariance structure is estimated in which diagonal terms are constrained to have binomial variance and off-diagonal terms are estimated (Yang et al. 2000). In this way, the dependence of observations at this level is fully accounted. By unconstraining level 2 variances, the assumption of binomal distribution may be assessed. Further assumptions were confirmed through analysis of residuals generated by the software (*MLwiN* 2.0, Centre for Multilevel Modelling, Bristol University, Bristol, UK). To compare effects of the same explanatory variables over the experiment, joint tests for equality across examinations were carried out using approximate Wald statistics.

Results

Two volunteers assigned to the test group declined further participation after week 4 because of examination stress. Clinical periodontal conditions of the study population at the outset and alterations 6 weeks after introducing experimental toothpastes are presented in Table 1. The volunteers had mild or moderate plaque-induced gingival bleeding (gingivitis) with few sites with increased PPD of >4 mm at partially erupted third molars (no loss of attachment). Any loss of clinical attachment was due to few facial areas with gingival recession. There were no significant differences between groups neither at baseline nor at the end of the study. During the course of the study differences were small within groups although bleeding tendency slightly attenuated in both groups (Table 1).

Table 1. Overall clinical data (mean ± standard deviation) of volunteers at baseline and difference after 6 weeks of using test or control toothpastes (week 10)

Variable	Baseline		Difference at week 10	
	control ($n = 17$)	test ($n = 17$)	control ($n = 17$)	test ($n = 15$)
PPD (mm)	1.84 ± 0.24	1.76 ± 0.30	− 0.08 ± 0.24	− 0.04 ± 0.18
CAL (mm)	0.01 ± 0.01	0.01 ± 0.01	+0.00 ± 0.02	+0.00 ± 0.01
BI (0–2)	0.26 ± 0.10	0.20 ± 0.14	**− 0.06 ± 0.08**	− 0.05 ± 0.12
% BOP	23.8 ± 8.7	18.9 ± 12.7	**− 5.5 ± 7.3**	**− 5.7 ± 8.6**
PLI (0–3)	1.09 ± 0.42	1.09 ± 0.37	+0.00 ± 0.49	+0.09 ± 0.33
% Plaque	66.1 ± 19.0	64.4 ± 18.8	− 1.8 ± 22.9	+2.5 ± 15.2
% CLS	1.9 ± 2.6	3.4 ± 3.7	**+4.6 ± 4.4**	+2.7 ± 6.3

Significant differences ($p < 0.05$) as compared with baseline are given in bold.
PPD, periodontal probing depth; CAL, clinical attachment level; BI, bleeding index (0–2); BOP, bleeding on probing; PLI, plaque index (0–3); CLS, calculus.

Ecological analysis of correlation between bleeding and PLI

Table 2 describes the correlations between individual's mean plaque and bleeding indices. Significant correlations were noticed in the preparatory phase (weeks 0–4) as well as in the control group thereafter. During the experimental phase (weeks 6–10), correlations were no longer significant in the test group.

Typical examples of the correlation between mean PLI and mean BI can be seen in Figs 2 and 3. At the end of the preparatory phase, a significant correlation was calculated (Fig. 2). Six weeks after introducing the experimental toothpaste, a similarly steep regression line is present in the control group but not in the test group (Fig. 3).

Multivariate multilevel models

Marginal quasi-likelihood and first-order approximation procedure provided converged estimates for all models. Table 3 presents fixed part estimates from a multivariate four-level model of BOP during the preparatory phase. Any increase in PLI by one score increased the odds of BOP by 34–44%

Table 2. Correlation (Pearson's *r*) between individual's mean plaque and bleeding index in subjects using test or control toothpaste after week 4

	Combined groups		Control		Test	
	r	*p*	*r*	*p*	*r*	*p*
Baseline	0.31	0.072				
Week 2	**0.49**	0.003				
Week 4	**0.37**	0.033				
Week 6			0.40	0.112	−0.12	0.667
Week 8			**0.56**	0.021	0.31	0.226
Week 10			**0.59**	0.013	0.10	0.712

Significant correlations ($p<0.05$) are given in bold.

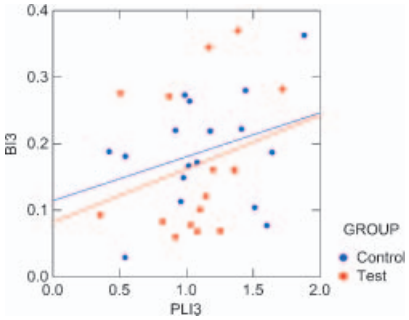

Fig. 2. Correlation between mean plaque (PLI3) and bleeding index (BI3) at the end of the preparatory phase in test and control groups.

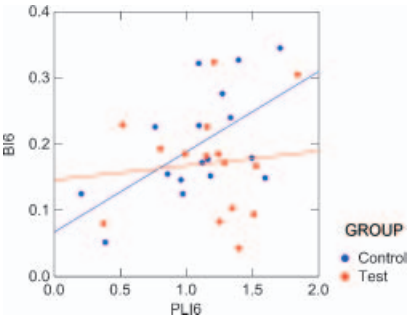

Fig. 3. Correlation between mean plaque (PLI6) and bleeding index (BI6) 6 weeks after introducing the experimental toothpaste in test and control groups.

(ORs between exp(0.292) = 1.34 and exp(0.368) = 1.44). Likewise, with any 1 mm increase of PPD the odds of BOP increased by about 60%. CLS was also positively associated with BOP. Infrequent attachment loss prevented entertaining CAL in the model. The random part of the model is presented in Table 4. At the site level, biserial correlations for BOP were very low (between 0.11 and 0.13). At the tooth level, correlations were larger (about 0.3), and highest at the subject level (0.7–0.9). Estimated extrabinomial parameters for the three binary responses were below 1, pointing to some underdispersion at the site level. Constraining the model to fit binomial variation at the lowest level did not substantially alter any of the other parameter estimates (not shown).

The final examination of the preparatory phase was included as a second baseline examination in a multivariate four-level variance components model for BOP during the experimental phase (Table 5). There was a gradually increasing negative effect of test toothpaste on BOP proportions. At the end of the experimental phase, the odds of BOP was decreased by 30% in the test group [OR 0.71%, 95% confidence interval (CI) 0.56–0.90, $p = 0.005$]. Standardizing the study population by introducing experimental toothpastes reduced subject variation from 0.6 to below 0.2 (Table 6).

Multivariate multilevel models were also built with PLI and CLS as responses (not shown). The test toothpaste had no significant, in particular no reducing, effect on PLI. There was a small, albeit not significant, negative effect on CLS. Owing to highly localized occurrence of CLS, the model revealed considerable underdispersion at the site level, making the assumption of Binomial distribution questionable. When level 3 (tooth) was removed from the model, extrabinomial variation was essentially reduced. In contrast to bleeding scores, biserial correlations for CLS were high (about 0.6) at the site level.

In Table 7, associations between BOP and different PLI scores in test and control group as derived from a multivariate four-level model are listed. At the end of the experimental phase associations between BOP and plaque scores were lower in the test group as compared with those in the control group ($\chi^2_3 = 8.39$, $p<0.05$).

Table 3. Fixed effects estimates (standard errors in brackets) of multivariate four-level model of bleeding on probing during preparatory phase

Parameter	Baseline ($t = 1$)	Week 2 ($t = 2$)	Week 4 ($t = 3$)
$\beta_{0,t}$	−2.622 (0.142)	−2.728 (0.149)	−2.020 (0.170)
PLI (0–3)	0.320 (0.038)	0.368 (0.040)	0.292 (0.041)
PPD (mm)	0.493 (0.048)	0.437 (0.052)	0.467 (0.052)
CLS (0, 1)	0.430 (0.190)	0.295 (0.158)	0.434 (0.151)

See Table 1 for explanation of abbreviations.

Adverse effects

One volunteer of the test group developed at the end of the experimental phase a painless geographic tongue (benign migratory glossitis), which disappeared after termination of the experiment. None of the other volunteers complained about adverse effects of toothpastes.

Discussion

In 16 controlled clinical trials with at least 6 months duration and enrolling between 54 and 329 subjects with plaque-induced gingivitis (Davies et al. 2004), mean plaque and gingivitis scores were consistently lower in subjects using triclosan/copolymer-containing toothpaste than in subjects using fluoride-containing toothpaste. In these studies averages of subject's means for plaque and gingivitis indices were provided. In the present study, Table 2 lists correlations calculated between summary measures of plaque and bleeding scores at different time points. It might be concluded from these results that the relationship between both variables becomes weaker after introducing triclosan-containing toothpaste. However, analysis of the correlation of mean values is prone to the so-called ecological fallacy, and generalization of observations to the site level can mislead or even be invalid, as the site-specific (''causal'') relationship is not properly taken into account. A further serious disadvantage of analyzing summary measures is immediate loss of most of the information usually acquired during examination of numerous periodontal

Table 4. Random part of multivariate four-level (occasion, site, tooth, subject) model of bleeding on probing during preparatory phase

	Parameter	Baseline ($t = 1$)	Week 2 ($t = 2$)	Week 4 ($t = 3$)
Level 4 (subject)	$\sigma^2_{f_t}$	0.323 (0.088)	0.363 (0.099)	0.585 (0.154)
	$\sigma_{f_{t,t+1}}$	0.304 (0.085) (0.888)	0.335 (0.105) (0.726)	
	$\sigma_{f_{t,t+2}}$	0.379 (0.106) (0.871)		
Level 3 (tooth)	$\sigma^2_{v_t}$	0.266 (0.056)	0.333 (0.063)	0.509 (0.067)
	$\sigma_{v_{t,t+1}}$	0.096 (0.043) (0.336)	0.107 (0.047) (0.260)	
	$\sigma_{v_{t,t+2}}$	0.124 (0.044) (0.336)		
Level 2 (site)	$\sigma^2_{e_t}$	0.897 (0.018)	0.888 (0.018)	0.836 (0.017)
	$\sigma_{e_{t,t+1}}$	0.101 (0.013) (0.113)	0.116 (0.012) (0.134)	
	$\sigma_{e_{t,t+2}}$	0.094 (0.012) (0.109)		

No level 1 (occasion) variation, but at level 2 (site) binomial variates covary. Extra-binomial variation allowed. Standard errors are given in first and correlations in second brackets.

Table 5. Fixed effects estimates of multivariate four-level (occasion, site tooth, subject) model of bleeding on probing at final examination of the preparatory phase and during the experimental phase

Parameter	Week 4 ($t = 3$)	Experimental phase		
		Week 6 ($t = 4$)	Week 8 ($t = 5$)	Week 10 ($t = 6$)
β_0	− 2.650 (0.170)	− 2.779 (0.167)	− 2.629 (0.168)	− 2.872 (0.168)
Toothpaste*		− 0.235 (0.155)	− 0.263 (0.153)	− 0.341 (0.121)
PLI (0–3)	0.269 (0.040)	0.297 (0.043)	0.260 (0.043)	0.255 (0.044)
PPD (mm)	0.452 (0.051)	0.510 (0.056)	0.460 (0.055)	0.566 (0.056)
CLS (0, 1)	0.490 (0.149)	0.267 (0.158)	0.404 (0.157)	0.639 (0.151)

*Reference: control toothpaste.
See Table 1 for explanation of abbreviations.

sites (altogether more than 170,000 in the present study). Although site specificity of periodontal disease is well established, it still seems to be common practice to average even highly expensive and valuable laboratory data on a subject level to ensure the necessary assumption of independence of observations for more traditional statistical analyses. In contrast, multilevel modelling provides the opportunity to use all available information with correct estimates of standard errors within hierarchical data. It allows the correlation of responses at each level to be modelled. Another advantage of our approach is that it gives a direct way of looking at the influence of subjects' unobserved characteristics on the response variable and hence a direct measure of the effects which we are assessing. Certainly, marginal models (Diggle et al. 2002) can be used as an alternative to analyse correlated data, in particular when interest is mainly on the effect of explanatory variables on the individual level response, and correlation structure is considered a nuisance. To our knowledge, the present study is the first

Table 6. Random part of multivariate four-level variance components model of bleeding on probing during the experimental phase

	Parameter	Week 4 ($t = 3$)	Experimental phase		
			Week 6 ($t = 4$)	Week 8 ($t = 5$)	Week 10 ($t = 6$)
Level 4 (subject)	$\sigma^2_{f_t}$	0.600 (0.158)	0.304 (0.089)	0.344 (0.099)	0.173 (0.057)
	$\sigma_{f_{t,t+1}}$	0.315 (0.101) (0.737)	0.292 (0.085) (0.903)	0.219 (0.067) (0.898)	
	$\sigma_{f_{t,t+2}}$	0.365 (0.109) (0.804)	0.199 (0.062) (0.866)		
	$\sigma_{f_{t,t+3}}$	0.263 (0.082) (0.816)			
Level 3 (tooth)	$\sigma^2_{v_t}$	0.552 (0.067)	0.405 (0.073)	0.472 (0.074)	0.411 (0.076)
	$\sigma_{v_{t,t+1}}$	0.260 (0.051) (0.566)	0.066 (0.053) (0.150)	0.148 (0.054) (0.337)	
	$\sigma_{v_{t,t+2}}$	0.035 (0.051) (0.071)	0.178 (0.054) (0.437)		
	$\sigma_{v_{t,t+3}}$	0.111 (0.052) (0.240)			
Level 2 (site)	$\sigma^2_{e_t}$	0.830 (0.017)	0.902 (0.019)	0.895 (0.018)	0.922 (0.019)
	$\sigma_{e_{t,t+1}}$	0.119 (0.013) (0.138)	0.139 (0.013) (0.155)	0.125 (0.013) (0.138)	
	$\sigma_{e_{t,t+2}}$	0.130 (0.013) (0.151)	0.102 (0.013) (0.112)		
	$\sigma_{e_{t,t+3}}$	0.133 (0.013) (0.152)			

The final examination of the preparatory phase (4 weeks) is included in the model. Standard errors are given in first and correlations in second brackets.

Table 7. Associations between bleeding on probing and plaque index (PLI) scores (0–3, reference PLI0) at final examination of the preparatory phase (week-4) and during the experimental phase

	Week 4 ($t = 3$)	Experimental phase					
		Week 6 ($t = 4$)		Week 8 ($t = 5$)		Week 10 ($t = 6$)	
		control	test	control	test	control	test
PLI1	1.70 (1.41–2.05)	1.95 (1.52–2.52)	1.49 (1.11–1.99)	1.49 (1.17–1.92)	1.58 (1.21–2.07)	2.11 (1.65–2.71)	1.42 (1.06–1.92)*
PLI2	2.03 (1.72–2.38)	2.12 (1.71–2.63)	1.85 (1.47–2.33)	1.83 (1.47–2.27)	1.97 (1.57–2.47)	2.43 (1.97–3.00)	1.86 (1.48–2.33)*
PLI3	2.26 (1.42–3.58)	3.92 (2.20–6.97)	2.50 (1.18–5.28)	2.69 (1.43–5.04)	2.54 (1.19–5.45)	2.23 (1.08–4.61)	1.07 (0.40–2.87)*

*Joint test between test and control group at weeks 10, $\chi^2_3 = 8.39$, $p = 0.039$.
Odds ratios and 95% confidence intervals are given.
PLI, plaque index.

attempt to explore, at the site level, alterations of the ''causal'' relationship between supragingival plaque and gingival inflammation by introducing an antibacterial compound, triclosan, in toothpaste. In that regard, it is a true pilot study, as it was not clear whether the association is actually affected by this, evidently also anti-inflammatory, agent at all. In order to get an idea about the required sample size for studying the site-specific attenuation of plaque and inflammation relationship in a steady-state plaque environment, which might be described by an OR for presence of plaque of, say, 2.5 (Müller et al. 2002), a difference between ln-transformed ORs of 0.5 was considered clinically relevant. If the standard deviation of ln-transformed ORs is also 0.5, 17 volunteers in each arm are required to detect the relevant difference with a less than 5% type I error and 80% statistical power. However, study design and duration, as well as data analysis differed considerably in this and the former study (Müller et al. 2002), and respective calculations have to be regarded tentative. In fact, no data exist so far, which might describe the possible degree of site-specific attenuation of the ''causal'' association between plaque and gingival inflammation.

This study was intentionally confined to young adult female non-smokers, which demands a more in-depth discussion. First, the correlation between mean proportions of gingival units BOP and tooth surfaces covered with plaque is lower in smoking periodontitis patients or dental hospital admissions (Bergström & Boström 2001), and quitting smoking may reverse this effect (Nair et al. 2003). In a representative sample, heavy smoking had a strong suppressive effect on gingival bleeding (Dietrich et al. 2004). Smoking reduces the bleeding tendency of gingiva in response to bacterial plaque in gingivitis experiments (Danielsen et al. 1990, Lie et al. 1998). In longitudinal studies, heavy tobacco consumption may lead, at site level, to gingival bleeding unrelated to the amount of supragingival plaque (Müller et al. 2002) pointing to smoking as an independent risk factor for gingival inflammation. Since it was anticipated that smoking largely confounds the site-specific association, any smokers were excluded from the study population. Second, gender differences have been reported in a recent gingivitis experiment (Waschul et al. 2003). Females showed, besides differences in gingival crevice cytokine profile, a reduced bleeding response to plaque at rest but an increased response under psychological stress. Also, sex hormone fluctuations during menstruation may have subtle effects on the relationship between plaque and gingival inflammation (Machtei et al. 2004), although reported differences were generally very low. In order to reduce variability (Shearer et al. 2005) the present study was therefore conducted in female non-smokers, which certainly limits the generalizability of the results.

Before introducing experimental toothpastes, the steady-state situation was studied in a 4-week preparatory phase, during which volunteers were advised not to alter their oral hygiene practices. It has recently been claimed that even the simulated participation in a clinical trial on experimental toothpaste may lead to significant improvements in personal oral hygiene (Feil et al. 2002), a phenomenon which can be attributed to the well-known Hawthorne effect. In the present study, mean PLI (1.09–1.12) and BOP proportions (19–21%) did not change in either direction during the preparatory phase. In a multivariate four-level model of BOP scores, associations between BOP and PLI (ORs between 1.34 and 1.44 per mm score increase), and BOP and PPD (ORs between 1.55 and 1.64 per mm increase in PPD) were highly consistent during the preparatory phase (see Table 4). Thus, a potential Hawthorne effect on plaque levels, bleeding scores, and site-specific associations between these variables can be considered minor. There are no obvious reasons for lack of any Hawthorne effect in the present study, but cultural peculiarities may play a role.

The multivariate multilevel logistic model allows for strong dependence between successive outcomes but does not require long series of repeated measures. The dependence is modelled by the covariance structure at the lowest (site) level, in which the diagonal terms are constrained to have binomial variance and off-diagonal terms, i.e. biserial covariances, are estimated. Here, diagonal parameters were less than 1, suggesting some extrabinomial variation, in this case underdispersion. Among possible reasons, Wright (1997) identified, in a simulation study, sparseness of data at one or more lower levels as the major source of underdispersion. In the present study, teeth defined a distinct level with six observations on each tooth. If the tooth level was removed, extrabinomial variation largely attenuated. The problem of including teeth, as a separate level in oral studies should be addressed in future studies.

Biserial covariances for BOP between successive examinations were very low, about 0.1. It has been shown that, provided the marginal probability of the outcome does not change across successive examinations, this covariance translates to the minimum conditional probability of the same outcome in successive exams (Griffiths et al. 2004). The corresponding low correlations are not surprising since repeat probing even within 24 h leads to highly variable bleeding results in subjects with plaque-induced gingivitis (Müller & Barrieshi 2005). During the preparatory phase volunteers reported usage of altogether 16 different toothpastes (brand names) and were even allowed to change the product from one examination to the other. Standardization by introducing two experimental (test and control) toothpastes reduced BOP variation at the subject level drastically from 0.6 immediately before to below 0.2 at the end of the experimental phase (see Table 6). After introducing experimental toothpastes, estimated probabilities for BOP gradually decreased in the test group. In addition, the site-specific (''causal'') association between BOP and PLI scores became weaker in the test group. The effect was strongest for the highest PLI score 3 (see Table 7). Notably, these effects developed gradually and were significant only at the end of the 10-week experiment. Dampening effects on gingival inflammation, and quantitative or even qualitative (Rosling et al. 1997) changes in the related microbiota may take time to manifest themselves in clinically visible alterations. In some studies, triclosan-related reductions of plaque and/or gingivitis have been reported only after extended periods of usage (Bruhn et al. 2002). It is most likely that respective effects may be more consistent after longer usage only. A potential effect of higher content of fluoride in control toothpaste on gingival bleeding tendency and the association between plaque and BOP could not be ascertained.

Within the limits of this study the following can be concluded: (I) 6 weeks after introducing 0.3% triclosan/2%

copolymer-containing toothpaste, the correlation between mean PLI and bleeding scores was apparently attenuated in young female non-smokers, while introduction of fluoride-containing toothpaste without triclosan did not alter this relationship. (II) Whereas the likelihood of BOP at the site level was decreased by the test toothpaste, PLI and presence of CLS were not affected in this short-term study. (III) A site-specific attenuation of the causal association between PLI scores and BOP in subjects using triclosan in toothpaste could already be discerned after 6 weeks. (IV) Not at least, multilevel modelling of the abundance of observations usually acquired in periodontal examinations in subjects has definitely the potential to provide deeper insights into pathogenetic and therapeutic mechanisms at the level of interest, the periodontal site, while concomitant consideration of random parameters, i.e. variances and covariances, at the site, tooth, and subject levels is allowed as well.

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Clinical Relevance

Scientific rationale for study: To study the effect of triclosan-containing toothpaste on the relationship between plaque and gingival bleeding in gingivitis and to investigate the potential of appropriate statistical methods for using all site-specific data collected in clinical trials to obtain definitive conclusions in small sample populations and after shorter time.

Principal findings: Whereas the likelihood of BOP at the site level was decreased already after 6 weeks of using triclosan-containing toothpaste, plaque levels and presence of CLS were not affected. A site-specific dampening of gingival inflammation in subjects using triclosan in toothpaste can already be discerned after 6 weeks.

Practical implications: Multilevel modelling of site-specific data in periodontal examinations has great potential to provide deeper insights into pathogenetic and therapeutic mechanisms at the level of interest, the periodontal site, while concomitant consideration of random parameters, i.e. variances and covariances, at the site, tooth, and subject levels is allowed as well.