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Effects of triclosan on host response and microbial biomarkers during experimental gingivitis

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Abstract

Aim: This exploratory randomized, controlled clinical trial sought to evaluate anti-inflammatory and -microbial effects of triclosan during experimental gingivitis as assessed by host response biomarkers and biofilm microbial pathogens.

Materials and Methods: Thirty participants were randomized to triclosan or control dentifrice groups who ceased homecare for 21 days in an experimental gingivitis (EG) protocol. Plaque and gingival indices and saliva, plaque, and gingival crevicular fluid (GCF) were assessed/collected at days 0, 14, 21 and 35. Levels and proportions of 40 bacterial species from plaque samples were determined using checkerboard DNA-DNA hybridization. Ten biomarkers associated with inflammation, matrix degradation, and host protection were measured from GCF and saliva and analysed using a multiplex array. Participants were stratified as "high" or "low" responders based on gingival index and GCF biomarkers and bacterial biofilm were combined to generate receiver operating characteristic curves and predict gingivitis susceptibility.

Results: No differences in mean PI and GI values were observed between groups and non-significant trends of reduction of host response biomarkers with triclosan treatment. Triclosan significantly reduced levels of *A. actinomycetemcomitans* and *P. gingivalis* during induction of gingivitis.

Conclusions: Triclosan reduced microbial levels during gingivitis development (ClinicalTrials.gov NCT01799226).

Gingivitis is a reversible inflammatory periodontal disease associated with dental plaque. It appears in high prevalence, with estimations of more than half of the United States' adults. (Loe et al. 1978, Eke et al. 2012).

The classic model of experimental gingivitis (EG) was developed in 1965 by Löe et al. and is routinely used today to study the pathogenesis and microbiologic changes from health to gingivitis (Loe et al. 1965). Most recently, this model has been adapted to examine changes in oral fluid inflammatory biomarkers (Lee et al. 2012). This study identified changes in 10 inflammatory biomarkers and 40 pathogens during induction and resolution of gingivitis (EG). They also stratified subjects based on their clinical inflammatory response and used baseline levels of salivary biomarkers to predict that

response. Others have since confirmed these findings demonstrating changes in gingival crevicular fluid (GCF) biomarkers with induction of gingivitis using this model or the traditional EG model (Heasman et al. 1993, Offenbacher et al. 2007, 2010, Salvi et al. 2010, Trombelli et al. 2010, Farina et al. 2012, Scott et al. 2012, Eberhard et al. 2013).

Triclosan is a preservative and broad-spectrum anti-microbial agent (Jones et al. 2000). It has significant anti-inflammatory properties by inhibiting the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (Muller et al. 2006) as well as host-derived inflammatory mediators such as interleukin (IL) 1β, IL-6, tumour-necrosis factor, and prostaglandins (PG) (Modeer et al. 1996, Barros et al. 2010, Trombelli & Farina 2013). Triclosan also significantly reduces plaque, calculus, and gingivitis, especially in patients who have pre-existing established gingivitis (Hioe & van der Weijden 2005, Panagakos et al. 2005, Muller et al. 2006, Teles & Teles 2009, Vered et al. 2009, Riley & Lamont 2013). Additionally, when used in conjunction with tooth brushing, it has also been shown to reduce *Fusobacteria* and *Veillonella* (Arweiler et al. 2002, Fine et al. 2006) and to inhibit IL-1β, IL-6, and PGE$_2$ (Modeer et al. 1996, Panagakos et al. 2005, Muller et al. 2006, Barros et al. 2010).

Stent models have been utilized to evaluate anti-inflammatory effects of triclosan dentifrice during EG. While Saxton and colleagues found superior reductions in plaque formation and gingivitis with triclosan as compared to a control (Saxton & van der Ouderaa 1989), others were unable to demonstrate such clinical differences (Saxton et al. 1993, Lang et al. 2002). These studies, however, only focused on clinical parameters and contained dentifrice formulations that are not currently available.

The aim of this exploratory study was to assess the anti-inflammatory effects of triclosan dentifrice compared to a control during experimental gingivitis. The primary outcome was to examine GCF biomarker analytes. Periodontal clinical measurements, microbial analysis, and whole saliva biomarker analyses were also performed.

Materials and Methods

Study population

Participants provided written informed consent to the protocol approved by the University of Michigan Health Sciences Institutional Review Board before administration of research-related procedures. The investigation was conducted at the Michigan Center for Oral Health Research in accordance with the Helsinki Declaration of 1975, as revised in 2000. The inclusion and exclusion criteria of the study population are outlined in Appendix S1.

Clinical procedures

Figure 1 summarizes the study timeline. A double-blind, controlled, parallel group study modelling the EG protocol originally described by Löe and Theilade was performed (Loe et al. 1965). At screening (day −14) a periodontal evaluation, dental prophylaxis, and oral hygiene instruction were performed (*n* = 78). Full-mouth PD, recession (REC), CAL, and BOP were recorded using a periodontal probe at six sites per tooth on all teeth except the third molars. Participants provided a urine specimen to confirm their non-smoking status. Pregnancy status was obtained through questionnaires given at each study visit. Mandibular alginate impressions were taken and customized stents were made by a single examiner based on a modification of a previously described study (Saxton & van der Ouderaa 1989). Appendix S2 provides a description of the stent fabrication. At day 0 (baseline), if all inclusion and exclusion criteria were met, participants entered the experimental phase of the trial. Participants who met all inclusion criteria but had a BOP score of >10% were given additional oral hygiene instruction and returned in 2 weeks for a second assessment. Participants not reaching a BOP score of ≤10% by the second confirmation visit were not enrolled in the study.

Using an online randomization chart, participants were assigned to either the test (triclosan dentifrice) or control (fluoride dentifrice) arm of the study as well as to either the right or left stent side. During the induction phase (i.e., baseline to 21 days), participants were instructed to refrain from all hygiene procedures in the stent area. During this time period, participants delivered a total of 2 ml of their assigned dentifrice into their stent, allowing it to come into contact with the areas of EG for 2 min. twice daily while traditional tooth brushing was performed in the non-stent areas. A complete description of participant homecare instructions can be found in Appendix S3.

The test dentifrice was composed of 0.24% sodium fluoride 1100 ppm 0.243% (0.14% w/v fluoride ion) and triclosan 0.30% in combination with 2% polyvinyl methyl ether maleic acid copolymer as the active ingredients along with inactive ingredients (Colgate® Total® Clean Mint Paste, Piscataway, NJ, USA). The control dentifrice was composed of 0.76% sodium monofluorophosphate 1000 ppm (0.15% w/v fluoride ion) as the active ingredient along with inactive ingredients (Colgate® Cavity Protection Great Regular Flavor Fluoride Toothpaste). Both dentifrice types were packaged into identical white laminated tubes so as to ensure appropriate masking.

At 21 days, participants received a dental prophylaxis and reinstatement of homecare oral hygiene procedures from 21 to 35 days. All study materials were collected and compliance was assessed by calculating the amount of dentifrice used (Almerich et al. 2005). Re-establishment of gingival health was confirmed at day 35.

At days 0, 14, 21, and 35, the following data were collected in this sequence: whole saliva, intra-oral photos, GI, PI, plaque samples, and GCF. Gingival index (GI) (Loe & Silness 1963) and plaque index (PI) (Silness & Loe 1964) were taken from the stent quadrant Plaque and GCF samples were collected from the stent quadrant using a randomization chart to ensure no site was sampled twice. Repeat GCF samples were collected at 1, 2, 4, and 6 h post-brushing at 14 and 21 days.

Five examiners underwent training and calibration procedures for PD, REC, CAL, BOP, PI, and GI prior to study initiation. Agreement was reached within 1 mm or better

Study Timeline and CONSORT Flow Chart

Screening Phase

Experimental Gingivitis Phase

Recovery Phase

Day -14
- Clinical measures
- Prophylaxis
- OHI
- Impression for stent
- Cotinine urine analysis

Day 0
- BOP
- Saliva
- Photos
- Gingival index
- Plaque index
- Plaque collection
- GCF
- Randomization of study arm, stent side, and GCF/plaque collection sites
- Stent instructions

Day 14
- Saliva
- Photos
- Gingival index
- Plaque index
- Plaque collection
- GCF *
- Patient incentive

* Samples collected before tooth brushing and at 1,2,4,6 hours after tooth brushing

Day 21
- Saliva
- Photos
- Gingival index
- Plaque index
- Plaque collection
- GCF *
- Prophylaxis
- Stent collected
- Unused study dentifrice collected
- Patient incentive

* Samples collected before tooth brushing and at 1,2,4,6 hours after tooth brushing with stent

Day 35
- Saliva
- Photos
- Gingival index
- Plaque index
- Plaque collection
- GCF
- Patient incentive

580 Interested in Study

78 Consented & Screened

30 Enrolled In Study

Control Arm
N = 15

1 withdrew after BL
1 disqualified at Day 14

1 Subject Added

14 completed study

Treatment Arm
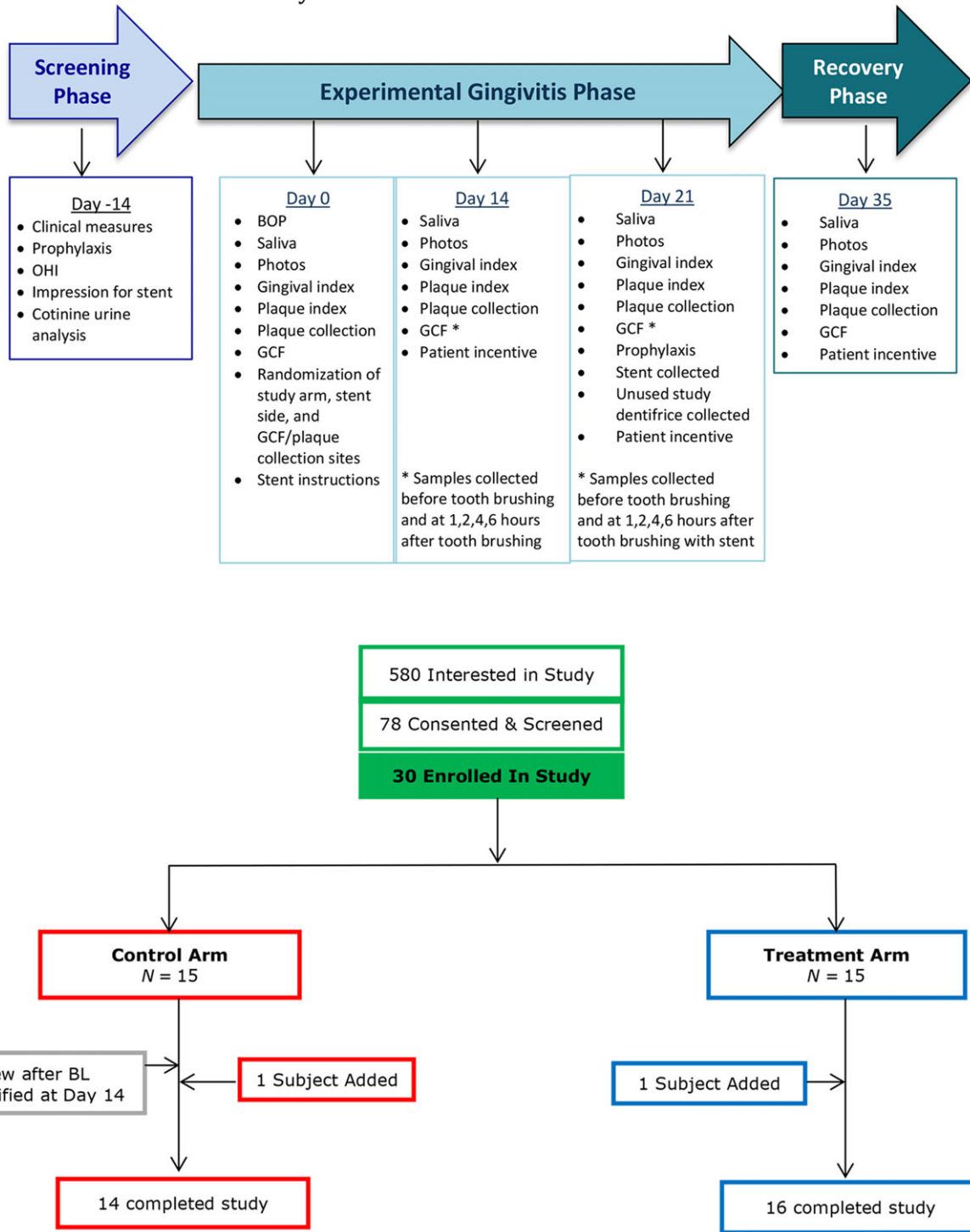*N* = 15

1 Subject Added

16 completed study

Fig. 1. Study timeline and recruitment and enrolment results of the study.

for measures of PD, REC, and CAL. For CAL measures, the mean inter-examiner correlation coefficient (using SPSS, Armonk, NY, USA) was 0.478 and the mean intra-examiner correlation coefficient (using SPSS) was 0.648. For PI and GI,

rather a pre-study visual and tactile examiner training was performed. During the study, every attempt was made to partner the examiner with the same patient. Both participants and examiners were masked to the randomization scheme.

Whole saliva collection and analysis

Unstimulated whole saliva was collected at the beginning of each visit and processed as previously described (Kinney et al. 2011). Inflammatory biomarkers were

quantified using a custom human 10-complex protein array that was optimized for sensitivity, specificity, stability, and intra-assay coefficient of variation by comparing to single cytokine enzyme-linked immunosorbent assays (Quantibody Custom Array, RayBiotech, Norcross, GA). Based on the results from Lee and colleagues, the 10-biomarker panel consisted of: IL-1α, IL-1β, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1, MMP-8, MMP-9, TIMP-1, and TIMP-2 (Lee et al. 2012). Appendix S4 provides additional information of the array procedures. Immediately after whole saliva collection, intra-oral photos were taken.

Microbial plaque collection and analysis

Supra- and sub-gingival plaque samples were collected using a sterile curette from two sites within the stent area. A randomization chart ensured that each site in the quadrant was sampled only once. Samples were placed into vials containing 150 μl of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, and 500 ml distilled water [pH 7.6]). 100 μl of 0.5 M NaOH was added into each vial. Samples were stored at 4°C until processed. Appendix S5 provides additional information regarding microbial analysis. Detection of 40 bacterial species was evaluated by the checkerboard DNA-DNA hybridization technique originally described by Socransky & Haffajee 1994, (Socransky & Haffajee 1994, Lee et al. 2012). The species evaluated and their corresponding microbial complexes are listed in Fig. S1.

GCF collection and analysis

Gingival crevicular fluid samples were collected at all study time points from the same two sites as the microbial plaque samples were taken. At 14 and 21 days, GCF samples were collected, then patients were instructed to place the dentifrice in the stent and brush the rest of their teeth for a 2 min. unsupervised session. Repeat GCF samples were collected from the same sites at 1, 2, 4, and 6 h post-brushing. A methylcellulose strip (Pro Flow, Inc., Amityville, NY, USA) was kept in place for 30 s and GCF was collected as previously described (Giannobile et al. 1995, Lamster & Ahlo 2007). Following collection, strips were kept on dry ice for transport and then stored at −80°C until analysis. The samples were thawed and proteins were extracted by an elution method (Giannobile et al. 1995). A complete description of the analysis procedures can be found in Appendix S6. Ten biomarkers were analysed based on the results from Lee and colleagues: IL-1α, IL-1β, IL-6, IL-8, IL-10, MCP-1, MMP-8, MMP-9, TIMP-1, and TIMP-2 (Lee et al. 2012).

Statistical analyses

See Appendix S7 for Comprehensive Statistical Analyses performed in this exploratory investigation.

Results

Figure 1 shows the CONSORT flow chart of participant recruitment and enrolment. Thirty participants completed the study; 14 in the control arm (2 males and 12 females; mean age: 27.1 ± 5.2 years) and 16 in the test arm (7 males and 9 females; mean age 26.1 ± 5.2 years). Table 1 depicts clinical and demographic information of the patients. No significant differences were found between the two groups in terms of race, age, and clinical parameters ($p > 0.05$).

Clinical changes with induction of gingivitis

Participants presented with high BOP scores at the screening visit and significantly reduced BOP scores at the baseline visit ($p < 0.001$) with no significant differences between the two groups. During the induction phase, significant increases in PI and GI were observed in both groups ($p < 0.001$) (Fig. 2a,b). A statistically significant difference was detected between groups at 14 days whereby the test group had increased plaque accumulation as compared to the control group ($p = 0.017$). All clinical parameters returned to baseline values at day 35, with significant differences seen from 21 days to 35 days in both groups ($p < 0.001$).

Microbial changes with induction of gingivitis

Total bacterial counts and counts of each microbial complex, except for the grey complex, increased significantly from baseline to 21 days and decreased significantly from 21 to 35 days ($p < 0.05$) (Fig. 3a,b).

A. actinomycetemcomitans was statistically significantly reduced in the test group as compared to controls from baseline to 14 days ($p = 0.013$) and from baseline to 21 days ($p = 0.038$). Statistically significant differences were also noted for *P. gingivalis*, which was markedly depressed by the triclosan dentifrice from baseline to 14 days as compared to control ($p = 0.006$).

GCF and salivary biomarker changes with induction of gingivitis

Both groups demonstrated mirrored and paralleled trajectories (Fig. 4).

Table 1. Participant demographics and clinical measures

Characteristics	Test (frequency)	Control (frequency)	*p*-value
Gender	16 (100%)	14 (100%)	NSD*
Male	7 (43.8%)	2 (14.3%)	
Female	9 (56.3%)	12 (85.7%)	
Race	16 (100%)	14 (100%)	NSD*
African American	1 (6.3%)	1 (7.1%)	
Asian	2 (12.5%)	2 (14.3%)	
Caucasian	11 (68.8%)	9 (64.3%)	
Hispanic	1 (6.3%)	1 (7.1%)	
Other	1 (6.3%)	1 (7.1%)	
Age, mean (SD)	26.06 (5.23)	27.07 (5.15)	0.60[†]
BOP at day −14 (SD)	0.47 (0.11)	0.49 (0.13)	0.66[†]
BOP at day 0 (SD)	0.09 (0.04)	0.10 (0.06)	0.62[†]
CAL at day −14, mean (SD)	0.76 (0.25)	0.75 (0.34)	0.93[†]

NSD, no significant difference.
*Fisher's exact test.
[†]Independent samples *t*-test.
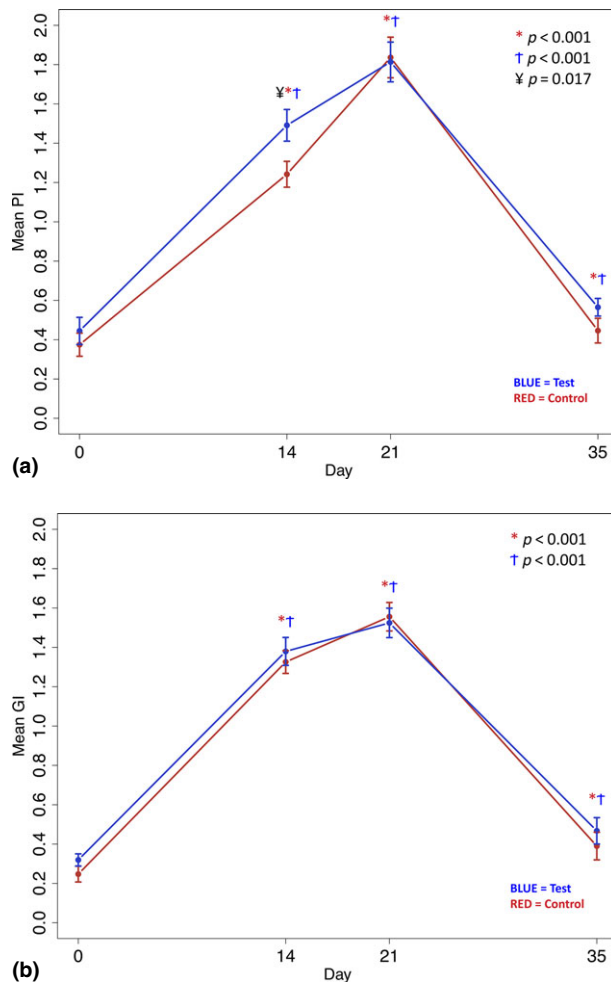
(a)

(b)

Fig. 2. A statistically significant increase in the plaque index and gingival index was noted with gingivitis induction as well as a statistically significant decrease with resolution ($p < 0.001$). At day 14, the test group had a statistically significantly higher plaque index compared to the control group ($^{¥}p = 0.017$).

GCF IL-1α and IL-1β increased significantly from baseline to 21 days ($p < 0.05$) and decreased significantly during the recovery period for both groups. IL-8 and MCP-1 were significantly reduced during induction ($p < 0.05$) and increased during resolution ($p < 0.05$). TIMP-1 and TIMP-2 were significantly increased with resolution of gingivitis only in the test group ($p < 0.05$).

Though there were no significant differences at any hourly time points or areas under the curve (AUC) between the groups at 14 days or 21 days ($p > 0.05$) (Figs S2 and S3), several trends were noted.

No significant differences in salivary biomarkers were noticed over time or between dentifrice groups for any of the biomarkers (Fig. S4).

Prediction of gingivitis response

Sixteen participants belonged to the "high" responder group (9 test participants and 7 control), whereas 14 participants belonged to the "low" responder group (7 test participants and 7 control). IL-1β was the best single marker with an AUC of 0.64. The best pair combination was IL-1β and MMP-8 with an AUC of 0.71. The best trio combination was IL-1β, MMP-8, and F. *nucleatum ss polymorphum* with an AUC of 0.85 with an odds ratio of 2.96 (95% CI= 1.16, 7.57) of being a "high" responder (Fig. 5).
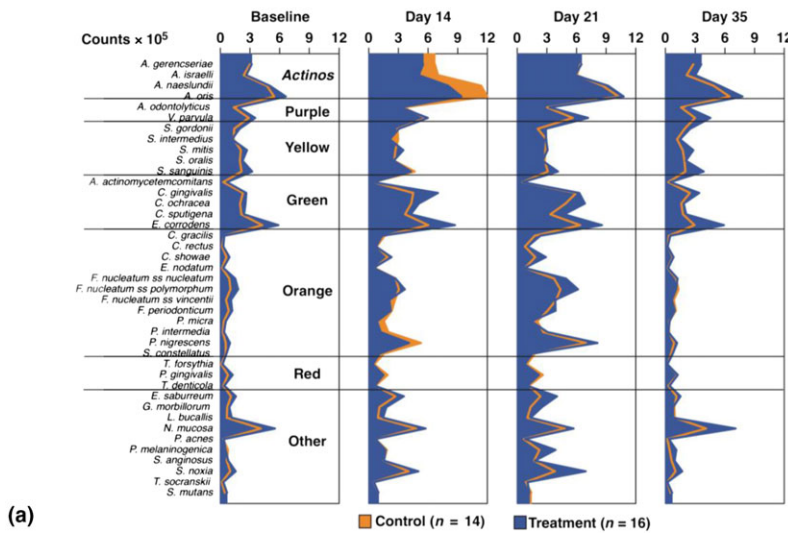
Discussion

The aims of this exploratory study were to assess how biomarkers and

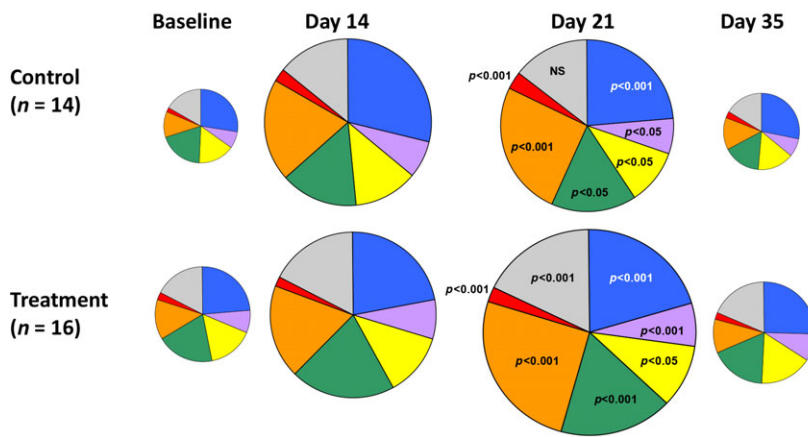pathogens are modulated by triclosan dentifrice when used during induction of EG.

All patients developed gingivitis, with similar increases in GI and PI to previous studies (Loe et al. 1965, Offenbacher et al. 2010, Salvi et al. 2010, Lee et al. 2012). Our inability to demonstrate significant clinical differences between groups remains consistent with one study (Lang et al. 2002), yet contrasts with several other studies (Saxton et al. 1993). Vered et al. demonstrated remarkable effects of triclosan over control dentifrices when used for 6 months or longer (Vered et al. 2009), though a different study reported improved anti-gingivitis activity as early as 6 weeks (Panagakos et al. 2005). The underestimated actions of triclosan in our study may be related to limitations in our study design, specifically, our rigid inclusion criteria and small sample. As such, the effects of triclosan may not have been as dramatic as when used in a situation beginning with poorer oral hygiene, for example. It has been reported that participants with higher baseline gingival bleeding scores were more likely to show reductions in bleeding and inflammation using a triclosan dentifrice in a 3-month clinical trial (Shearer et al. 2005).

Interesting patterns of GCF biomarker results emerged, especially when comparing the study day (0, 14, 21, 35) longitudinal findings to repeat sampling at 0, 1, 2, 4 and 6 h post-dentifrice exposure. As expected, overall IL-1β levels increased in both groups during the induction phase of the study (day 0 to 21). Our results mirror those previously reported in other EG studies (Zhang et al. 2002, Trombelli et al. 2006, and Salvi et al. 2010). However, a marked drop in both IL-1α and IL-1β concentrations was observed in both the test and controls groups at 14 days and 21 days 1 h post-dentifrice application. Furthermore, a continued sustained reduction in IL-1α levels was seen for 6 h post exposure. Regarding IL-8 production, our results are similar to others (Zhang et al. 2002, Deinzer et al. 2007, Lee et al. 2012) and showed a short-term down-regulation of this biomarker, especially early in the early stages of experimental gingivitis. What is notewor-

Mean counts (×105) of Microbial Species Stratified by Dentifrice Type

(a)

□ Control (*n* = 14)   ■ Treatment (*n* = 16)

Pie Chart Demonstrating Progression and Proportions of Each Microbial Complex in Relation to Total DNA from Day 0 to Day 35 Stratified by Dentifrice Type

(b)   P value calculated using Friedman test for changes over time.

Fig. 3. Blue line delineates bacterial counts in the test group. Orange fill represents bacterial counts in the control group.

hours after using the therapeutic agent. At 14 days, triclosan demonstrated trends of depressing levels of MMP-8 and -9 and TIMP-1 at 2 and 4 h after application through the stent. It was also able to maintain these depressed levels over the 6 h after application in MMP8 and TIMP-1. Furthermore, triclosan increased TIMP-2 levels just hours after its application on 21 days as demonstrated by the AUC analysis. This clearly demonstrates that triclosan has an immediate effect (within hours) in depressing levels of enzymes that destroy collagen in the early stages of gingivitis induction. In addition, after the depressed effects on the matrix degradation enzymes, triclosan was then able to increase the concentration of their inhibitors, further protecting the periodontium from destruction.

Based on our current knowledge, this is the first study demonstrating the anti-microbial effects of triclosan through DNA-DNA hybridization when used as a locally applied agent without the use of mechanical tooth brushing. The test group was found to have statistically significantly depressed changes in counts of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans,* as well as demonstrated trends for depressed levels of *Tannerella forsythia.* Changes in *Actinomyces species* were also found to be depressed, although not statistically significant, in the test as compared to the control. These species are commonly associated with developing gingivitis (Loesche & Syed 1978, Slots 1979, Mombelli et al. 1990). The test group also demonstrated a trend for decreased levels of *Peptostreptococcus micra* and *Fusobacterium nucleatum.* *P. micra* has been shown to increase in gingivitis (Slots 1979, Mombelli et al. 1990) chronic periodontitis, and active progressive periodontal disease (Rams et al. 1992). Furthermore, its elimination has been associated with clinical improvement (Haffajee et al. 1988). *F. nucleatum* is a key component of the biofilm as it possesses the ability to coaggregate with multiple species in the oral cavity and bridge primary colonizers found in gingivitis with secondary colonizers such as *P. gingivalis* found in periodontitis (Yang et al. 2014).
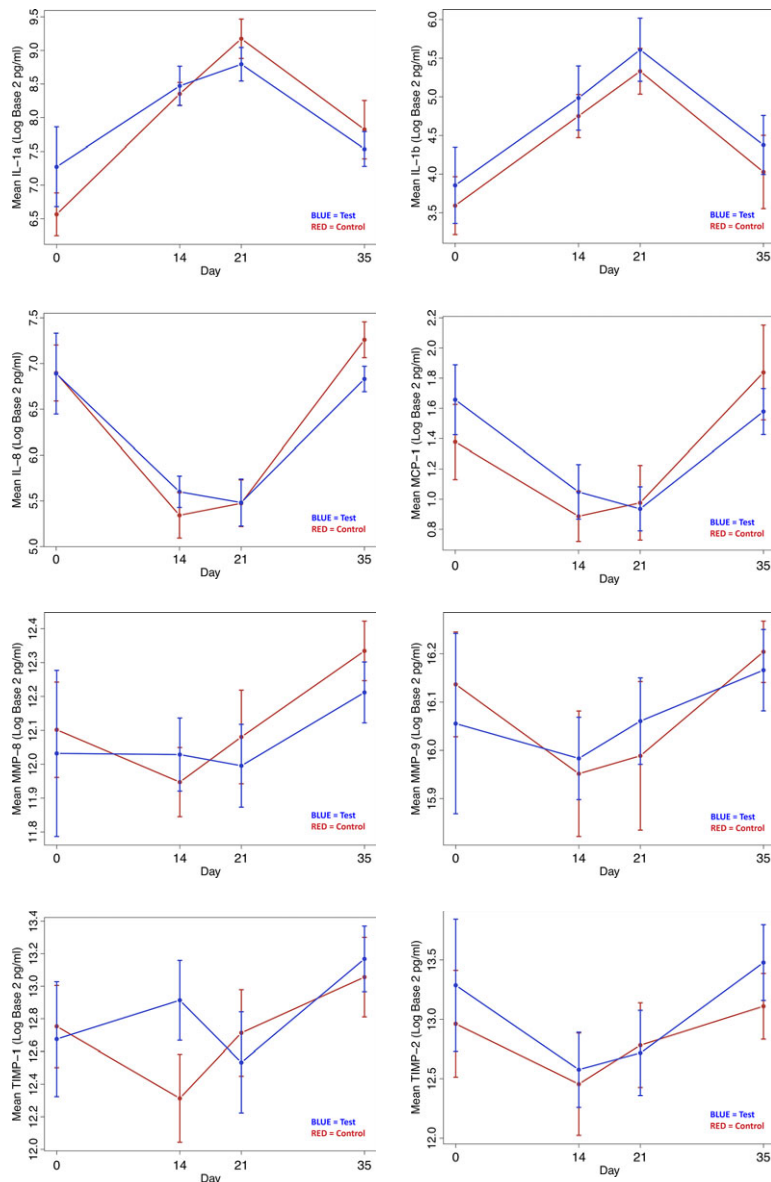
thy is that our repeat IL-8 GCF sampling results at 14 days and 21 days showed a strong increase in this cytokine up to 2 h post-dentifrice application. After 2 h of dentifrice exposure levels of IL-8 appeared to plateau off, but were still elevated from pre-application concentrations. Other GCF biomarker differences were observed between the mean study day levels and the repeated sampling levels at 14 and 21 days. For example, while mean levels of MCP-1 decreased from day 0 to 14 and 21 days, post-dentifrice contact levels 1, 2, 4 and 6 h steadily rose. A similar trend was seen in the mean levels of TIMP-2. There was an overall decrease in the concentrations of this biomarker during the induction phase of the study; yet repeat post-dentifrice contact levels rose at 14 and 21 days.

The uniqueness of the model permitted evaluation of the effects of triclosan in the absence of tooth brushing and eliminated participant differences in oral hygiene practices as a potential bias. Furthermore, the model demonstrated differences in biomarker concentrations within

Fig. 4. GCF IL-1α and IL-1β significantly increased with gingivitis induction (IL-1α; *$p < 0.001$, †$p = 0.005$) (IL-1β; *$p < 0.001$, †$p = 0.004$) and decreased with resolution (IL-1α; *$p = 0.006$, †$p < 0.001$) (IL-1β; *$p = 0.020$, †$p = 0.033$). IL-8 and MCP-1 significantly decreased with gingivitis induction (IL-8; *$p < 0.001$, †$p = 0.004$) (MCP-1; *$p = 0.046$, †$p = 0.004$) and increased with resolution (IL-8; *$p < 0.001$, †$p < 0.001$) (MCP-1; *$p = 0.039$, †$p = 0.0.03$). No significant differences in matrix degradation enzymes were observed. TIMP-1 and TIMP-2 significantly increased with resolution of gingivitis in the test group (TIMP-1; †$p = 0.020$) (TIMP-2; †$p = 0.009$).

Our findings remain consistent with former studies that demonstrate the benefit of triclosan dentifrice over a control when used in conjunction with tooth brushing (Arweiler et al. 2002, Fine et al. 2006). In this study we see declines in these microbial reductions, however, they were found to diminish considerably when meticulous patient home care and professional debridement was performed (Teles & Teles 2009). It is possible that trends and non-significant differences were noted in our study due to the small patient sample and extremely meticulous oral hygiene required by the participants prior to cessation of oral hygiene. Had the patients entered the experimental phase of the study with high levels of plaque and inflammation, it is possible that the effects of treatment may have been more noticeable. It is also possible that the anti-microbial and anti-inflammatory functions of triclosan may have been limited as a result of its placement and possible absorption into a thick, experimentally induced plaque filled surface. As such, we propose that the active chemical components, triclosan and fluoride, remain effective when used in conjunction with tooth brushing.

Baseline values of the combination of IL-1β, MMP-8, and *F. nucleatum ss polymorphum* had the best ability to discriminate between "high" *versus* "low" responders. Doubling baseline values of IL-1β while controlling for MMP-8 and *F. nucleatum ss polymorphum* is associated with 3x the odds of displaying an exaggerated inflammatory response compared to those with low levels of at least one of these markers. MMP-8 was also shown to play an important role in predicting the inflammatory response. While our study did not demonstrate remarkable changes in this biomarker with gingivitis induction, others have shown an increase in MMP-8 with cessation of oral hygiene (Offenbacher et al. 2010, Salvi et al. 2010). Additionally, *F. nucleatum* as a bridging species has been found to predominate in gingivitis (Listgarten 1976, Slots 1979, Mombelli et al. 1990). In fact, Lee and colleagues also determined that this microbe predisposed participants to be at higher risk of gingivitis (Lee et al. 2012). Thus, "high" responders possess a specific microbial profile and immuno-inflammatory phenotype at baseline, which predicts colonization of periodontal pathogens and inflammatory cytokines.

Our exploratory study identified changes in host immune and microbial biomarkers that transpire during the pathogenesis of gingival inflammation. Though our pilot study was unable to demonstrate superior clinical effects, we did detect depressed levels in microbes and inflammatory GCF biomarkers during gingivitis development with the use of triclosan applied through a stent model. The test group demonstrated an increased ability to reduce *Actinomyces species*, red complex pathogens, *A. actinomycetemcomitans*, and bridging organisms *F. nucleatum* and *P. micra* with induction of gingivitis.
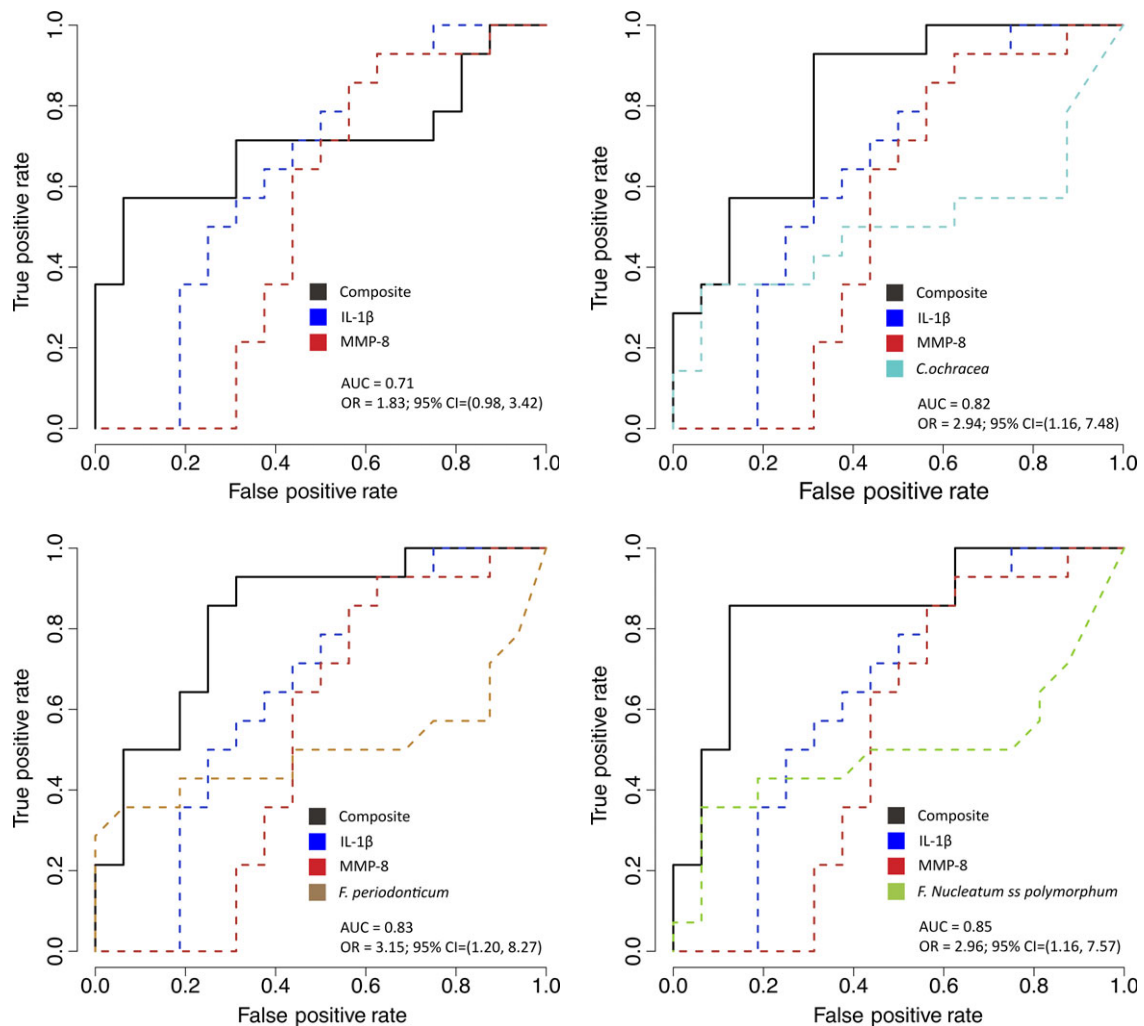
Fig. 5. The composite of IL-1β, MMP-8, and *F. nucleatum ss. Polymorphum* generated an AUC of 0.85. Doubling baseline values of IL-1β while controlling for MMP-8 and F. *nucleatum ss polymorphum* is associated with 2.96 times the odds of being a "high responder" compared to participants with low levels.

Triclosan also depressed levels of MMPs within a few hours after triclosan application early on in gingivitis development along with an increase in protective inhibitors of matrix degradation enzymes at the later stages of gingivitis development. By identifying highly susceptible patients, early identification, diagnosis, and therapeutic intervention may be provided prior to irreversible attachment loss. Given the nature of this exploratory trial, future larger randomized controlled clinical trials should be directed towards investigating a cluster of biomarkers affected by gingivitis along with therapeutic modalities that best reduce their immuno-inflammatory and microbial component.

Conflict of Interest

Dr. William V. Giannobile has served as a consultant to Colgate, however not for this specific study. Dr. Foti Panagakos currently serves as Worldwide Director of Research Relations and Scientific Affairs, Colgate-Palmolive.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. 40 Microbial pathogens and their respective complexes analysed using the checkerboard DNA-DNA hybridization technique.

Fig. S2. No significant differences in pro-inflammatory cytokines were observed over time at day 14.

Fig. S3. There was a trend for higher IL-1β values at the 1 ($p = 0.087$) and 2 ($p = 0.076$) hour time points in the test group compared to the control at day 21.

Fig. S4. No significant differences in salivary biomarkers were observed.

Table S1. Microbial changes during experimental gingivitis model.

Appendix S1. Patient inclusion and exclusion criteria.

Appendix S2. Description of stent fabrication.

Appendix S3. Participant instructions for using the stent.

Appendix S4. Supplemental information about salivary biomarker array procedures.

Appendix S5. Supplemental informa-tion about checkerboard DNA-DNA hybridization techniques.

Appendix S6. Supplemental GCF analysis information.

Appendix S7. Statistical analyses.

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Clinical Relevance

Scientific rationale for the study: The effects of triclosan dentifrice on oral fluid biomarkers in the absence of mechanical plaque removal are unknown.

Principal findings: Local applica-tion of triclosan demonstrated an ability to reduce microbial patho-gens, of particular interest red com-plex pathogens and bridging organisms. It also lowered levels of matrix metalloproteinases early in the development of experimental gin-givitis while increasing levels of pro-tective inhibitors of collagen degradation enzymes later in the gingivitis state.

Practical implications: The use of a triclosan dentifrice may be consid-ered a therapeutic intervention to alter the oral microbiome in patients with gingivitis.