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Title: Effects of Triclosan on Host Response and Microbial Biomarkers during Experimental Gingivitis

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One sentence summary: Local application of triclosan demonstrates anti-microbial and anti-inflammatory properties in the early stages of experimental gingivitis

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 analyzed 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).

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 application of triclosan demonstrated an ability to reduce microbial pathogens, of particular interest red complex pathogens and bridging organisms. It also lowered levels of matrix metalloproteinases early in the development of experimental gingivitis while increasing levels of protective inhibitors of collagen degradation enzymes later in the gingivitis state.

Practical Implications:

The use of a triclosan dentifrice may be considered a therapeutic intervention to alter the oral microbiome in patients with gingivitis.

Introduction

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. (Eke et al., 2012, Loe et al., 1978).

The classic model of experimental gingivitis (EG) was developed in 1965 by Loe 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 ten inflammatory biomarkers and forty 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 (Eberhard et al., 2013, Farina et al, 2012, Heasman et al., 1993, Offenbacher et al., 2010, Offenbacher et al., 2007, Trombelli et al, 2010, Salvi et al., 2010, Scott et al., 2012).

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) 1 β , IL-6, tumor-necrosis factor, and prostaglandins (PG) (Trombelli et al., 2013, Barros et al., 2010, Modeer et al., 1996). Triclosan also significantly reduces plaque, calculus, and gingivitis, especially in patients who have pre-existing established gingivitis (Muller et al., 2006, Panagakos et al., 2005, Hioe et al, 2005, Riley and Lamont, 2013, Teles and Teles, 2009, Vered et al., 2009). 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-1 β , IL-6, and PGE₂ (Barros et al., 2010, Modeer et al., 1996, Muller et al., 2006, Panagakos et al., 2005).

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 and van der Ouderaa, 1989), others were unable to demonstrate such clinical differences (Lang et al., 2002, Saxton et al., 1993). 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 A*.

Clinical Procedures

Figure 1 summarizes the study timeline. A double-blind, controlled, parallel group study modeling the EG protocol originally described by Loe and Theilade was performed (Loe et al., 1965). At screening (day -14) a periodontal evaluation, dental prophylaxis, and oral hygiene instruction was 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 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 and van der Ouderaa, 1989). *Appendix B* 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 $\leq 10\%$ 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 21d), participants were instructed to refrain from all hygiene procedures in the stent area. During this time period, participants delivered a total of 2mL 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 C*.

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). 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 21d, participants received a dental prophylaxis and reinstatement of homecare oral hygiene procedures from 21d to 35d. All study materials were collected and compliance was assessed by calculating the amount of dentifrice used (Almerich et al., 2005). Reestablishment 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, intraoral photos, GI, PI, plaque samples, and GCF. Gingival index (GI) (Löe and Silness, 1963) and plaque index (PI) (Silness and Löe, 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 6h post-brushing at 14d and 21d.

Five examiners underwent training and calibration procedures for PD, REC, CAL, BOP, PI, and GI prior to study initiation. Agreement was reached within 1mm or better for measures of PD, REC and CAL. . For CAL measures the mean inter-examiner correlation coefficient (using SPSS) 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 and 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 intraassay 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-1 α , IL-1 β , 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 D* provides additional information of the array procedures. Immediately after whole saliva collection, intraoral 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 μ l of Tris-EDTA buffer (10mM Tris-HCl, 1 mM EDTA, and 500 mL distilled water [pH 7.6]). 100 μ l of 0.5 M NaOH was added into each vial. Samples were stored at 4°C until processed. *Appendix E* provides additional information regarding microbial analysis. Detection of 40 bacterial species was evaluated by the checkerboard DNA-DNA hybridization technique originally described by Socransky et al. 1994 (Lee et al., 2012, Socransky and Haffajee, 1994). The species evaluated and their corresponding microbial complexes are listed in *sFigure 1*.

GCF Collection and Analysis

GCF samples were collected at all study time points from the same two sites as the microbial plaque samples were taken. At 14d and 21d, GCF samples were collected, then patients were instructed to place the dentifrice in the stent and brush the rest of their teeth for a 2min unsupervised session. Repeat GCF samples were collected from the same sites at 1, 2, 4, and 6h post-brushing. A methylcellulose strip (Pro Flow, Inc., Amityville, NY) was kept in place for 30 seconds and GCF was collected as previously described (Giannobile et al., 1995, Lamster and Ahlo, 2007). Following collection, strips were kept on dry ice for transport and then stored in at -80° C until analysis. The samples were thawed and proteins were extracted an elution method (Giannobile et al., 1995). A complete description of the analysis procedures can be found in *Appendix F*. Ten biomarkers were analyzed based on the results from Lee and colleagues: IL-1 α , IL-1 β , 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 G* for Comprehensive Statistical Analyses performed in this exploratory investigation.

Results

Figure 1 shows the CONSORT flowchart of participant recruitment and enrollment. 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 & 2b). A statistically significant difference was detected between groups at 14d 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 21d to 35d 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 21d and decreased significantly from 21d to 35d ($p < 0.05$) (Figure 3a and Figure 3b).

A. actinomycetemcomitans was statistically significantly reduced in the test group as compared to controls from baseline to 14d ($p = 0.013$) and from baseline to 21d ($p = 0.038$). Statistically

significant differences were also noted for *P. gingivalis*, which was markedly depressed by the triclosan dentifrice from baseline to 14d 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). GCF IL-1 α and IL-1 β increased significantly from baseline to 21d ($p<0.05$) 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 14d or 21d ($p>0.05$) (sFig. 2 and sFig. 3), but several trends were noted.

No significant differences in salivary biomarkers were noticed over time or between dentifrice groups for any of the biomarkers (sFig. 4).

Prediction of Gingivitis Response

Sixteen participants belonged to the 'high' responders group (9 test participants and 7 control), whereas 14 participants belonged to the 'low' responders group (7 test participants and 7 control). IL-1 β was the best single marker with an AUC of 0.64. The best pair combination was IL-1 β and MMP-8 with an AUC of 0.71. The best trio combination was IL-1 β , 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 (Lee et al., 2012, Loe et al., 1965, Offenbacher et al., 2010, Salvi et al., 2010). 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 the 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 6h post-dentifrice exposure. As expected, overall IL-1 β 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-1 α and IL-1 β concentrations was observed in both the test and controls groups at 14d and 21d 1 hour post-dentifrice application. Furthermore, a continued sustained reduction in IL-1 α levels was seen for 6h 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 noteworthy is that our repeat IL-8 GCF sampling results at 14d and 21d showed a strong increase in this cytokine up to 2h post-dentifrice application. After 2h 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 14d and 21d. For example, while mean levels of MCP-1 decreased from day 0 to 14d and 21d, post-dentifrice contact levels 1, 2, 4 and 6 hours 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 14d and 21d.

The uniqueness of the model permitted evaluation of the effects of triclosan in the absence of toothbrushing and eliminated participant differences in oral hygiene practices as a potential bias. Furthermore, the model demonstrated differences in biomarker concentrations within hours after using the therapeutic agent. At 14d, triclosan demonstrated trends of depressing levels of MMP-8 and -9 and TIMP-1 at 2 and 4h after application through the stent. It was also able to maintain these depressed levels over the 6h after application in MMP8 and TIMP-1. Furthermore, triclosan increased TIMP-2 levels just hours after its application on 21d 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 and Syed, 1978, Mombelli et al., 1990, Slots, 1979). 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 (Mombelli et al., 1990, Slots, 1979) 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).

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 and 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 treatment may have been more noticeable. It is also possible that the antimicrobial 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-1 β , MMP-8, and *F. nucleatum ss polymorphum* had the best ability to discriminate between ‘high’ versus ‘low’ responders. Doubling baseline values of IL-1 β 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* is a bridging species has been found to predominate in gingivitis (Listgarten, 1976, Mombelli et al., 1990, Slots, 1979). 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* and with induction of gingivitis. 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 toward investigating a cluster of biomarkers affected by gingivitis along with therapeutic modalities that best reduce their immuno-inflammatory and microbial component.

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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**
* = Fisher's Exact Test, ** = Independent samples t-test, NSD = No significant difference			

Figure Legends:

Figure 1: Displays the study timeline and recruitment and enrollment results of the study.

Figure 2: A statistically significant increase in the plaque index and gingival index were 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 ($\text{¥} p = 0.017$).

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

Figure 4: GCF IL-1 α and IL-1 β significantly increased with gingivitis induction (IL-1 α ; * $p < 0.001$, † $p = 0.005$) (IL-1 β ; * $p < 0.001$, † $p = 0.004$) and decreased with resolution (IL-1 α ; * $p = 0.006$, † $p < 0.001$) (IL-1 β ; * $p = 0.020$, † $p = 0.033$). 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; * $p = 0.039$, † $p = 0.003$). 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).

Figure 5: The composite of IL-1 β , MMP-8, and *F. nucleatum ss. Polymorphum* generated an AUC of 0.85. Doubling baseline values of IL-1 β 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.

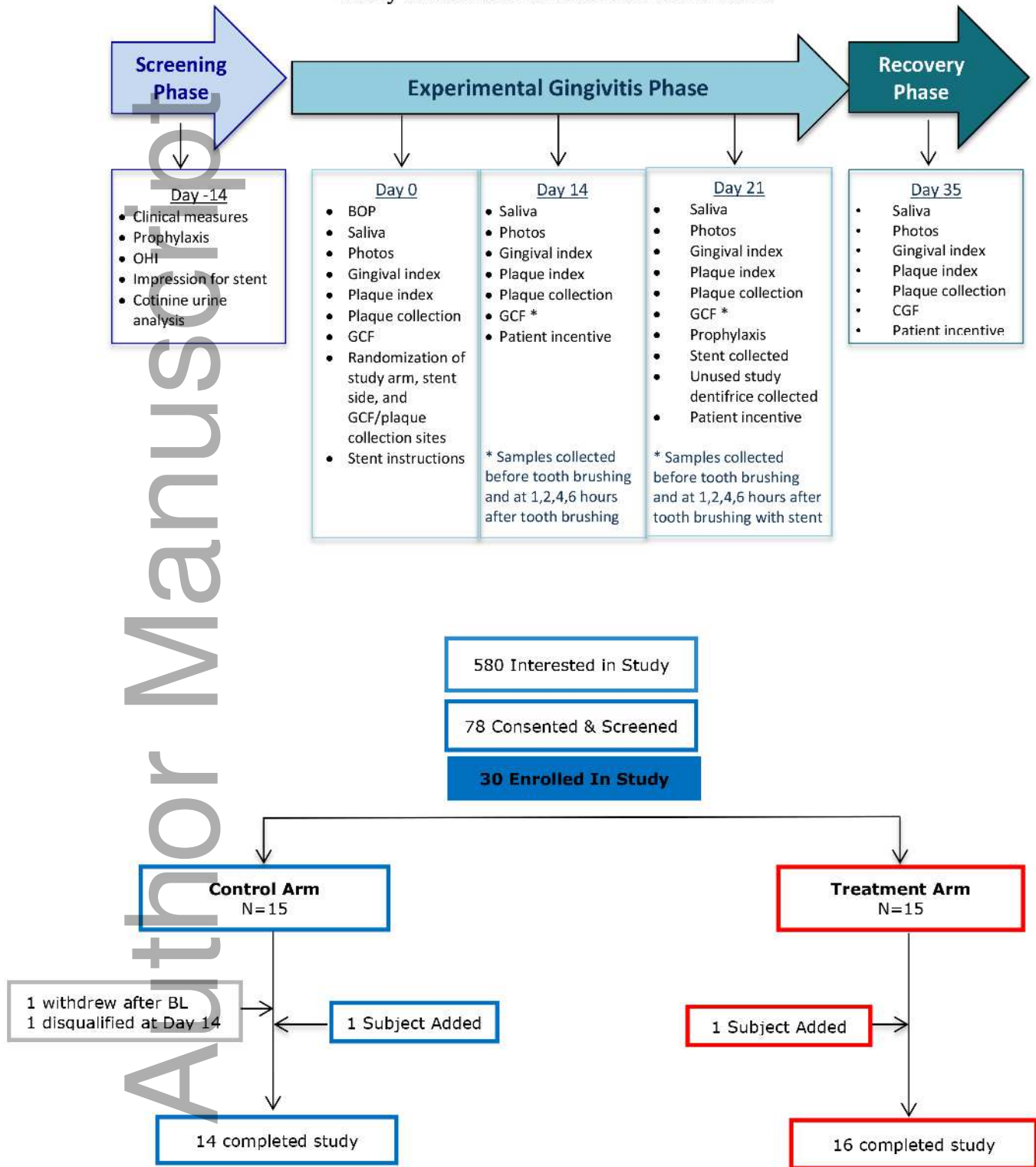
Supplemental Figure 1: 40 Microbial Pathogens and their Respective Complexes Analyzed using the Checkerboard DNA-DNA Hybridization Technique

Supplemental Figure 2: No significant differences in pro-inflammatory cytokines were observed over time at day 14. There was a trend for lower MMP-8 values at the 2 (p=0.096) and 4 (p=0.056) hour time points, MMP-9 values at the 2 (p=0.064) hour time point, and lower AUCs for both matrix degradation enzymes in the test group compared to the control at day 14. There was a trend for higher TIMP-1 values at the 0 (p= 0.099) hour time point at day 14.

Supplemental Figure 3: There was a trend for higher IL-1 β 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. No significant differences in matrix degradation enzymes were observed over time at day 21. There was a trend for higher TIMP-2 values at the 2 (p= 0.058) and 4 (p= 0.088) hour time points, and a higher AUC in the test group compared to the control at day 21.

Supplemental Figure 4: No significant differences in salivary biomarkers were observed.

Figure 1
Study Timeline and CONSORT Flow Chart



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Figure 2a
 Longitudinal Plot of Mean Plaque Index Stratified by Dentifrice Groups From Day 0 to Day 35

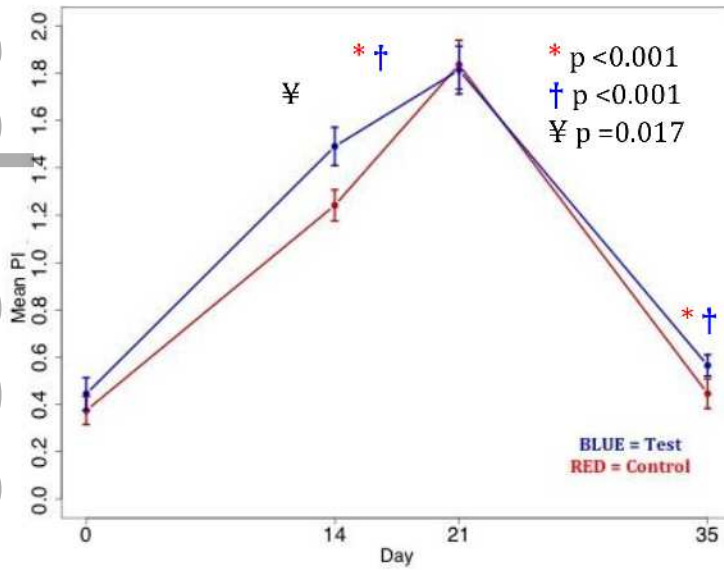
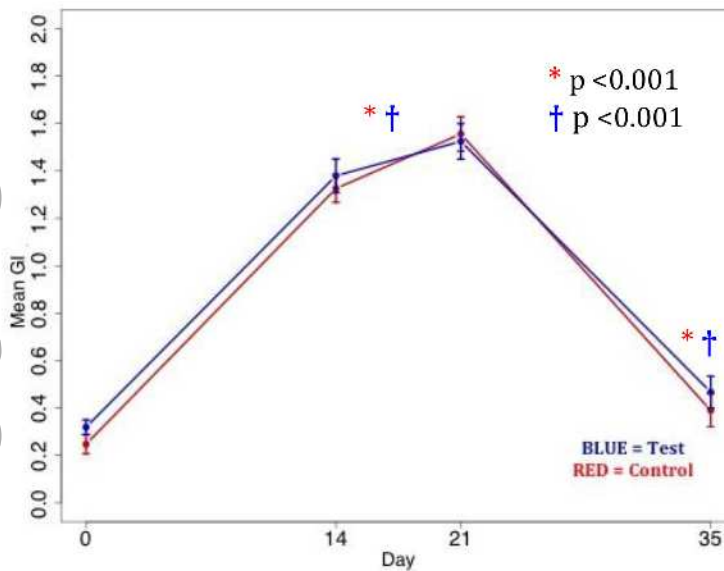


Figure 2b
 Longitudinal Plot of Mean Gingival Index Stratified by Dentifrice Groups From Day 0 to Day 35



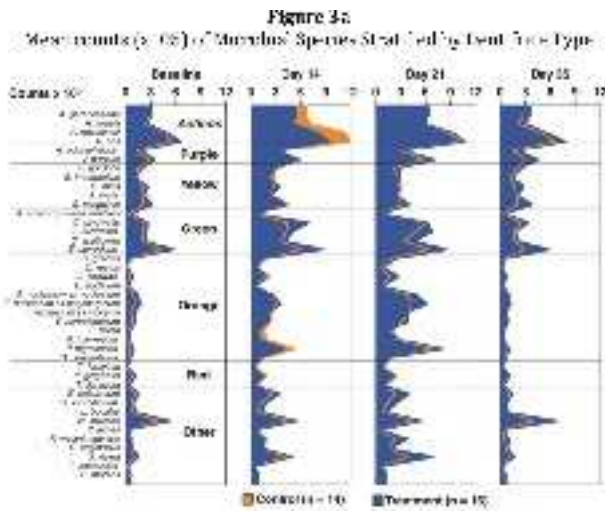
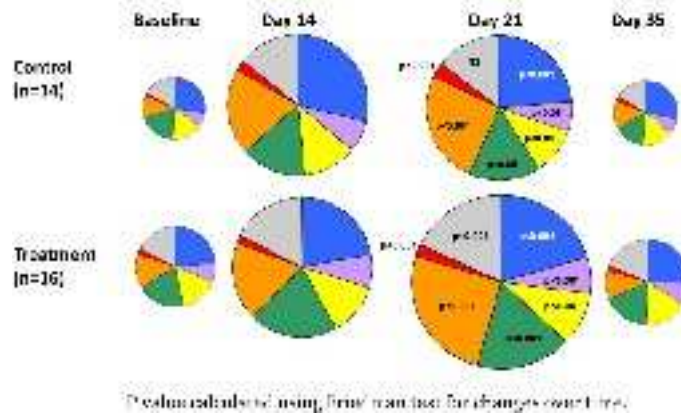


Figure 3b
 Pie Chart Demonstrating Progression and Proportions of Each Microbial Complex Relative to Total DNA from Day 0 to Day 35 Stratified by Denture Type



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Figure 4

Longitudinal Plots of Mean GCF Biomarkers (\pm SE) Stratified by Dentifrice Groups From Day 0 to Day 35; IL-1 α , IL-1 β , IL-8, MCP-1, MMP-8, MMP-9, TIMP-1, TIMP-2

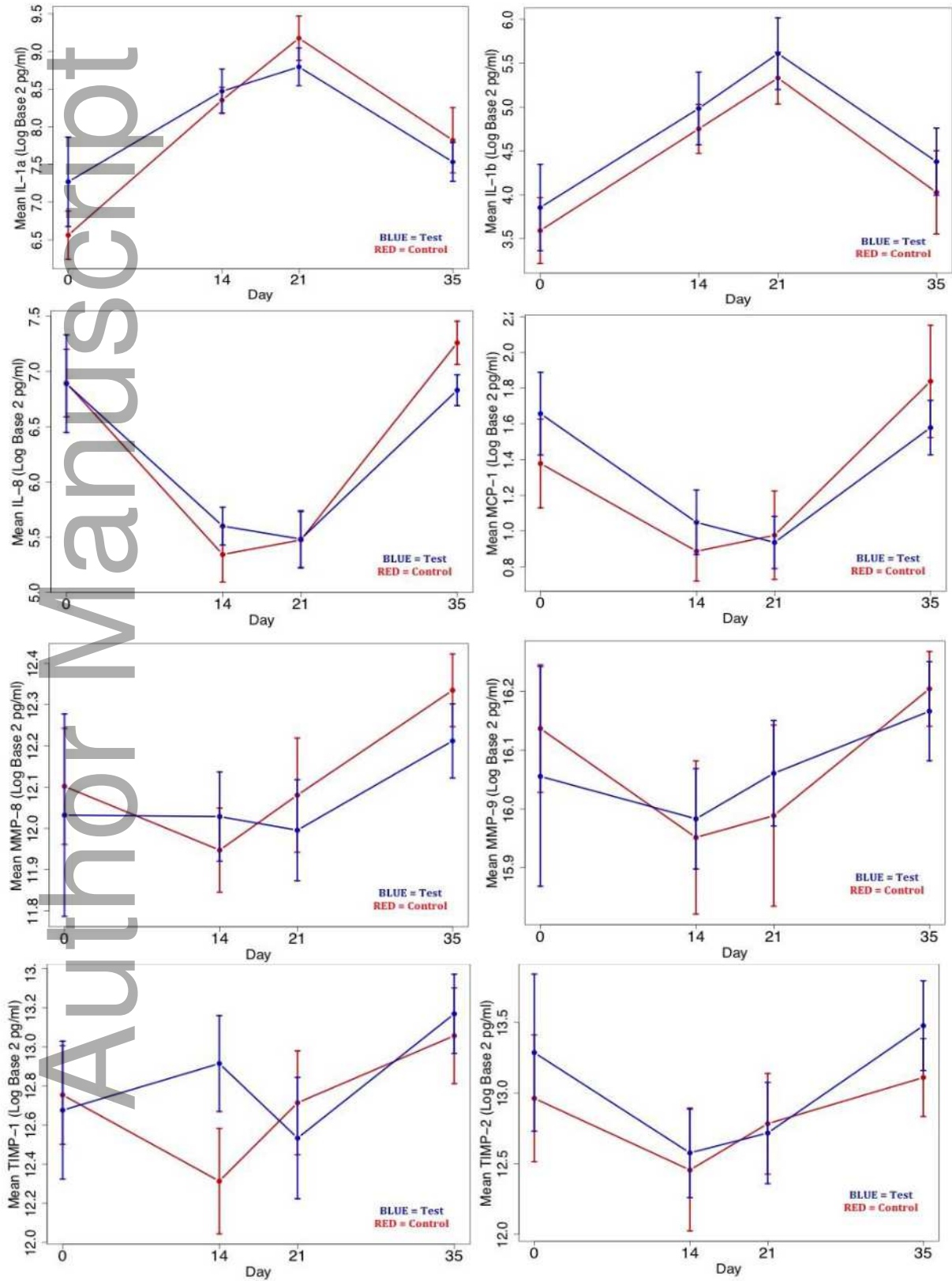
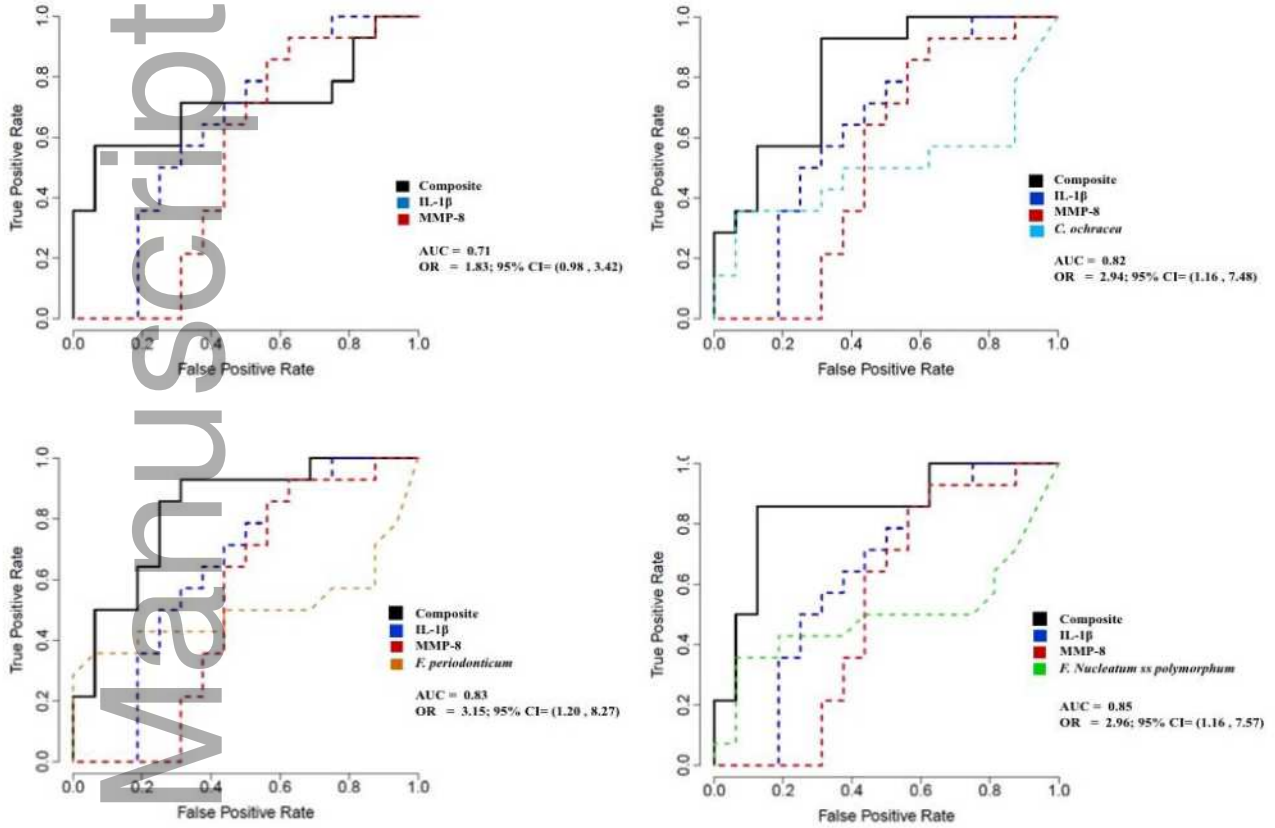


Figure 5

Receiver Operating Characteristic (ROC) Curves for GCF biomarkers IL-1 β , MMP-8 and Microbial Biomarkers *C. ochracea*, *F. periodonticum*, *F. nucleatum ss polymorphum* Predict High Gingival Index Over Time



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Author