Clinical effects of stannous fluoride dentifrice on peri-implant mucositis, plaque microbiome, and oxidative stress

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Abstract: Purpose: This single-center, single-treatment, 4-week study evaluated the efficacy of a stannous fluoride (SnF_2) dentifrice in reducing peri-implant mucositis while assessing changes in biomarkers and the oral microbiome profile. **Methods:** 24 healthy participants 18 years of age or older with osseointegrated implants were included, with 19 participants having mucositis and five without mucositis. The non-mucositis participants served as a reference group for microbiome and biomarker assessments (baseline comparison). All participants used the same 0.454% SnF_2 dentifrice (Crest Pro-Health Sensitive and Enamel Shield) and a soft manual toothbrush twice daily throughout the study. Participants received Modified Gingival Index (MGI) and Gingival Bleeding Index (GBI) assessments at Baseline - Day 1 and at Week 4 - Day 1. Subgingival plaque and peri-implant crevicular fluid (PICF) were collected on Baseline - Day 2 and Week 4 - Day 2 for oxidative stress markers, bacterial endotoxins, proinflammatory cytokines and 16S analysis. **Results:** After 4 weeks, participants with mucositis experienced a significant reduction from baseline in MGI and GBI scores (63.4% and 79.3%, respectively) and in the number of bleeding sites (72.5% based on GBI analysis). Microbiome analysis of subgingival plaque showed that mucositis was associated with a higher relative abundance of disease-associated genera (*Fusobacterium*, *Porphyromonas*, *Treponema*, and *Prevotella*) and a lower relative abundance of commensal genera (*Rothia* and *Actinomyces*). Alpha diversity was higher in the mucositis group compared to the non-mucositis group at baseline. By Week 4, the profile of participants with mucositis had shifted to align more closely with that of non-mucositis participants. Participants with mucositis showed significant reduction in biomarkers related to bacterial insult, plaque virulence, oxidative stress, and inflammation. Simulated pathway and process analysis revealed that multiple categories of genes were associated with a state of mucositis, and 4 weeks of use of the experimental dentifrice downregulated several virulence-associated genes. (*Am J Dent* 2025;38:59-66).

Clinical significance: In participants with mucositis, use of a SnF_2 dentifrice for 4 weeks reduced clinical signs and key biomarkers of peri-implant inflammation and shifted the oral microbiome toward a healthier profile, highlighting the utility of SnF_2 dentifrice in the control of peri-implant mucositis.

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Introduction

The prevalence of dental implants as the treatment of choice for teeth replacement is increasing, with projections indicating that 17% of adults with missing teeth will have a dental implant by 2026 based on recent trends.[1,2] As the number of dental implants increases, so does the problem of peri-implant mucositis, an inflammatory condition caused primarily by dysbiotic biofilm on the implant.[3-6] According to multiple systematic reviews, peri-implant mucositis affects 43% to 47% of dental implant patients.[7,8]

The microbial profile associated with peri-implant mucositis has similarities to that of periodontitis, with key bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* along with bacterial endotoxins (i.e., lipopolysaccharides) implicated in the progression of both diseases by triggering an inflammatory response.[9] Unresolved peri-implant mucositis can rapidly progress to peri-implantitis, an inflammatory condition characterized by bleeding, suppuration, mucosal recession, and bone loss.[3-5] Ultimately, peri-implantitis can result in the loss of the implant itself.[3] The rate of disease progression around implants is faster than around natural teeth,[10] driven by differences in bone/implant interface, mucosa, and vascularization around implants compared to natural teeth.[3] Studies suggest the inflammatory process around implants may be more severe and harder to reverse with treatment than is seen with natural dentition.[11] A study by Schierano et al[12] showed that reversibility of tissue inflammation to pre-experimental levels was possible after oral hygiene care was suspended and reinstituted, but it took three times as long as with natural teeth (69 days versus 21 days).

The significance and the role of reactive oxygen species (ROS) in oral disease has been well documented,[13-18] and ROS also plays a role in peri-implant disease progression. Prior research has demonstrated increasing levels of advanced glycation end products and lipid oxidation in peri-implant crevicular fluid (PICF) and saliva with the progression of peri-implant disease severity.[19-20] Myeloperoxidase (MPO), a protein enzyme that is a marker of inflammation and contributes to protease activity and connective tissue break-down, has been shown at higher levels in PICF around inflamed implant sites compared to healthy sites.[20-24] Thus, the analysis of PICF offers a non-invasive means of studying the host response in peri-implant disease and may provide an early indication of patients at risk for active disease. Moreover, analyses of PICF levels have shown significantly lower levels of interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8) and macrophage inflammatory protein-1alpha (MIP-1α) in healthy

patients with non-manifesting inflammation compared to early and late stages of mucositis.[25]

Progression from a state of health to a state of mucositis and finally to peri-implantitis and implant loss can be prevented with good oral hygiene. Peri-implant mucositis appears to be both preventable and treatable if the oral biofilm is restored to, and maintained at, healthful levels.[3-5] Stannous fluoride (SnF_2) dentifrices represent a potential self-care tool for the prevention and reversal of peri-implant mucositis. Clinical studies in non-implant populations have demonstrated that SnF_2 dentifrices quell plaque toxicity by blocking the activation of inflammation-triggering receptors,[26,27] limit plaque regrowth,[28] and reduce gingivitis,[29] an inflammatory condition which, like peri-implant mucositis,[3,4,6] is attributable to dysbiotic biofilm.[30,31] Research has also demonstrated that SnF_2 can reduce oxidative stress that leads to a hyper-inflammatory condition, resulting in cellular damage.[32] In addition to reducing overall quantity of plaque, SnF_2 dentifrices have been shown to promote a more healthful microbiome, inhibiting the growth of gingivitis-associated bacteria and promoting healthful genera.[33,34] However, there is a lack of published evidence on the effects of SnF_2 for peri-implant mucositis.

The present study was conducted to fill that gap by evaluating the efficacy of a SnF_2 dentifrice with respect to peri-implant mucositis, including reduction of clinical signs of inflammation; reduction of biomarkers of oxidative stress, plaque virulence, inflammation; and restoration of a healthful oral microbiome. This study also compared the oral microbiome profiles and simulated pathways of adults with peri-implant mucositis to those of adults with implants but no mucositis.

Materials and Methods

Design summary and ethics statement - This single-treatment, single-center pilot study was conducted over 4 weeks at Salus Research, Inc., Ft. Wayne, Indiana, USA. All subjects provided written, informed consent. The study protocol abided by all applicable laws, regulations, and guidelines (international, national, and institutional), including the US Code of Federal Regulations, the standards of the International Council for Harmonization Consolidated Guideline for Good Clinical Practice (ICH-GCP), and Procter & Gamble Standard Operating Procedures. The study protocol was approved by U.S.IRB (Miami, FL; U.S.IRB2022SRI/17).

Participants - Twenty-five healthy adult participants with at least one osseointegrated implant were targeted for enrollment: 20 participants with mucositis and five with no mucositis (reference group for microbiome and biomarker assessments). All enrollees were required to have at least 20 teeth and at least one tooth-bounded osseointegrated implant, in function for at least 1 year. Participants in the mucositis group were required to have bleeding upon probing of the implant; participants in the no mucositis group were required to have no bleeding upon probing and no swelling or redness associated with the implant.

Participants were excluded on the grounds of rampant caries, open or untreated caries, severe periodontal disease, or any signs of peri-implantitis (including suppuration or mobility). Exclusion was also made on the grounds of a participant having received dental prophylaxis within 3 months of a sampling visit or having taken antibiotics or used antigingivitis or antibacterial oral care products (e.g., SnF_2, chlorhexidine, or essential oils) within 2 weeks of plaque-sampling visits. Participation was excluded if antibiotics would be required as prophylaxis prior to dental visits or if any other disease or condition, or medication would be expected to interfere with experimental procedures or the safe completion of the study.

Study visit overview - The study was conducted over the course of four visits (Baseline - Day 1, Baseline - Day 2, Week 4 -Day 1, and Week 4 - Day 2). On the days before study visits, participants were required to refrain from eating, drinking, chewing gum, brushing their teeth, flossing, and using breath mints, beginning after the evening's brushing and continuing throughout the following day's study visit. Small sips of water were allowed up to 45 minutes prior to each visit.

At the Baseline - Day 1 visit, subjects provided written informed consent, and investigators collected medical history together with demographic information. Each participant received an oral and perioral examination of hard and soft tissues followed by MGI[35] and GBI[36] assessments. Participants received a periodontal exam to verify the presence or absence of implant-associated mucositis.

On Baseline - Day 2, an oral and perioral examination of hard and soft tissues was conducted. Supragingival plaque was removed, six PICF samples were taken from around each implant, and then subgingival plaque was collected. Following this, participants received treatment products together with written and verbal usage instructions and performed the first treatment brushing on site.

On Week 4-Day 1, participants each received an oral and perioral examination of hard and soft tissues, an MGI assessment, and a GBI assessment.

On Week 4-Day 2, an oral and perioral examination of hard and soft tissues was conducted. Supragingival plaque was removed, six PICF samples were taken from around each implant, and then subgingival plaque was collected.

Clinical evaluations - MGI score[35] was assigned at implant sites according to a scale ranging from 0 (no inflammation) to 4 (severe inflammation) and was assessed at six sites per implant. Scores were summed and divided by the total number of scorable sites to calculate an overall score. GBI score[36] was assigned at implant sites (at the buccal, mesial/distal, and lingual aspects of each implant) according to a scale ranging from 0 (no bleeding 30 seconds after probing) to 2 (immediate bleeding after probing). Scores were summed and divided by the total number of scorable sites to calculate an overall score. The number of bleeding sites reflects the number of sites with a GBI score of 1 or 2.

PICF sample collection - Supragingival plaque was removed with a scaler before PICF sampling, which followed a standard method: First, the test section was isolated with cotton rolls and the site was dried with an air stream. Then, cotton pliers were used to insert periopaper into each pocket for 30 seconds before the strip was then inserted into a vial with 200 µL of buffer, placed on dry ice, and stored at -70 °C until analysis.

PICF analysis - PICF was analyzed for markers of oxidative stress [carboxymethyl lysine (CML), lactate dehydrogenase activity (LDH) and myeloperoxidase (MPO)], bacterial endo-

Table 1. Baseline demographics.

Demographic	Mucositis (n=19)	No mucositis (n=5)	Overall (n=24)	P-value
Age (Years)				
Mean (SD)	46.16 (14.557)	56.20 (12.872)	48.25 (14.561)	0.1753[a]
Range	18 - 68	35 - 70	18 - 70	
Ethnicity				
Hispanic or Latino[b]	1 (5%)	0 (0%)	1 (4%)	1.0000[c]
Not Hispanic or Latino[b]	18 (95%)	5 (100%)	23 (96%)	
Race				
White/Caucasian[b]	19 (100%)	5 (100%)	24 (100%)	NA
Sex				
Female[b]	17 (89%)	4 (80%)	21 (88%)	0.5212[c]
Male[b]	2 (11%)	1 (20%)	3 (13%)	
Tobacco use				
Yes	0 (0%)	0 (0%)	0 (0%)	1.0000[c]
No	19 (100%)	5 (100%)	24 (100%)	

Abbreviations: NA, not applicable; SD, standard deviation.
[a] Two-sided ANOVA P-value for the treatment comparison.
[b] Data is presented as the number (percent) of subjects in each category.
[c] Two-sided chi-square P-value for the treatment comparison.

toxins, and proinflammatory cytokines (interferon [IFN]-γ, interleukin [IL]-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α). Volume of PICF was evaluated by Periotron[a] and the total amount of CML, LDH, MPO, cytokines, and endotoxins was calculated. Cytokines, endotoxins, and LDH were analyzed as described previously.[32,37] CML (Nε-(1-Carboxymethyl)-L-lysine) analysis was conducted using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry (HILIC-MS/MS). Briefly, 25 µL of each PICF sample and 250 µL of 6N HCl were added into a 4 mL glass vial. Samples were incubated at 110°C for 16 hours, dried with SpeedVac,[b] and then reconstituted with 100 µL diluent (0.1% formic acid in 50/50 water/acetonitrile) and 25 µL of internal standard (IS) solution (Nε-(1-Carboxymethyl)-L-lysine-d4: d4-CML). The analyte and internal standard were separated on an EMD Millipore ZIC-HILIC column[c] (2.1 × 150 mm, 5 µm) using a gradient between 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Detection and quantitation were by tandem mass spectrometry (AB Sciex 6500[d]) operating under positive electrospray ionization mode and multiple reaction monitoring (MRM) conditions (CML: 204.9 → 84.0; d4-CML: 209.0 → 88.0). The concentration of CML in the study specimens was determined from its corresponding peak area ratio (peak area analyte/peak area IS) by interpolation from a weighted ($1/x^2$) quadratic regression curve of calibration standards. The nominal range of quantitation was 0.1 ng/mL to 100 ng/mL. MPO activity was assessed with the myeloperoxidase assay kit purchased from Meso Scale Diagnostics.[e] PICF samples were diluted at 1:20 in PBS then equal volumes of diluted PICF samples, calibrators or controls were first added to each well. The rest of the assay procedures were performed following manufacturer's instruction. The concentrations were reported in pg/ml.

Subgingival plaque sample collection - After PICF sample collection, subgingival plaque was collected around implants using a sterile scaler avoiding contact with oral soft tissues. Plaque was transferred to labeled tubes with 200 µL of buffer, placed on dry ice, and stored at -70°C pending analyses of microbiome and biomarkers. A separate scaler was used to collect each sample. Plaque samples were transferred to ZymoResearch for DNA extraction and 16S analysis of the V3-V4 regions.

Subgingival plaque sample analysis - Toll-like receptor [TLR] 2 and TLR4 activity was analyzed as described previously.[32,37] 16S analysis was performed using taxonomic classification, KEGG[38] (Kyoto Encyclopedia of Genes and Genomes) gene, pathway functional analysis based on PICRUst simulation[39] with EZBiome[f] 16S Analysis software.[40]

Treatment - All participants received the same treatment: 0.454% SnF_2 toothpaste (Crest Pro-Health Sensitive and Enamel Shield Toothpaste[g]). Manual toothbrushes (Oral-B Indicator Soft Manual Toothbrush[g]) were provided to all participants for use throughout the study. Participants were instructed to brush twice daily (morning and evening) with the assigned dentifrice and toothbrush for 1 minute each time.

Safety - Adverse events were defined as unfavorable and unintended signs, symptoms, or diseases temporarily associated with, but not necessarily caused by, the experimental product.

Statistical analysis- The primary endpoint was the change from baseline in the number of implant-associated bleeding sites at Week 4 in participants with mucositis. Secondary endpoints were changes in microbiome and biomarker profiles as assessed in oral plaque and PICF at baseline and Week 4. The null hypothesis for assessments of the mucositis group was that posttreatment means would be equal to the respective means at baseline.

The sample size for this novel study was chosen for logistical considerations. Previous research[32] showed this sample size to be sufficient to detect improvement in participants with gingivitis who brushed with SnF_2 dentifrice after 4 weeks. Demographic and baseline clinical data were summarized. Comparisons of Week 4 means to baseline means were performed with paired-difference t-tests; all tests were two-sided, and analyses incorporated a 5% level of significance. Between-treatment comparisons also incorporated a 5% level of significance.

Microbiome data were analyzed with R software[h] version 4.1.3. Between-group differences in microbiome profiles were

Table 2. Clinical measurements in mucositis group (n=19).

Clinical measurement	Baseline	Week 4	Change from baseline[a]	Percent change from baseline[b]	P-value[c]
Mean (SD) MGI	2.79 (0.293)	1.02 (0.344)	1.77 (0.358)	63.44	<0.001
Mean (SD) GBI	0.58 (0.318)	0.12 (0.239)	0.46 (0.244)	79.31	<0.001
Mean (SD) no. of bleeding sites	3.63 (2.733)	1.00 (1.915)	2.63 (1.892)	72.45	<0.001

Abbreviations: GBI, Gingival Bleeding Index; MGI, Modified Gingival Index; SD, standard deviation.
[a] Change from baseline = baseline − week 4.
[b] Percent change from baseline = 100 × [(baseline day 1 − week 4 day 1) / baseline day 1].
[c] Tests mean change from baseline vs. 0; two-sided paired-difference test.

Table 3. Biomarker results for mucositis group.

Analyte	Geometric mean (CI) Baseline	Week 4	P-value
CML (PICF)	5.09 (3.79, 6.85)	3.53 (2.62, 4.75)	0.0001
Endotoxins (PICF)	1.04 (1.03, 1.04)	1.01 (1.00, 1.02)	<0.0001
IFN-γ (PICF)	4.18 (3.30, 5.30)	2.78 (2.20, 3.53)	<0.0001
IL-1β (PICF)	280.85 (179.24, 440.06)	218.76 (139.62, 342.78)	0.0401
IL-2 (PICF)	6.12 (4.55, 8.25)	6.16 (4.58, 8.30)	0.955
IL-4 (PICF)	1.77 (1.59, 1.98)	1.28 (1.15, 1.43)	<0.0001
IL-6 (PICF)	6.53 (4.88, 8.73)	4.79 (3.58, 6.40)	0.0008
IL-8 (PICF)	814.47 (618.96, 1071.73)	771.74 (586.49, 1015.51)	0.2811
IL-10 (PICF)	34 (16.14, 71.62)	7.55 (3.58, 15.89)	<0.0001
IL-12p70 (PICF)	2.28 (1.94, 2.64)	1.85 (1.60, 2.14)	0.0001
IL-13 (PICF)	11.84 (8.91, 15.75)	10.31 (7.75, 13.70)	0.0373
LDH (PICF)	1.26 (1.18, 1.35)	1.15 (1.07, 1.23)	0.0093
MPO (PICF)	1001.5 (567.23, 1762.04)	672.67 (382.33, 1183.5)	0.0018
TLR2 (Plaque)	1.51 (1.37, 1.66)	1.12 (1.02, 1.24)	<0.0001
TLR4 (Plaque)	1.14 (1.10, 1.18)	1.04 (1.01, 1.08)	<0.0001
TNF-α (PICF)	7.82 (5.74, 10.63)	7.28 (5.35, 9.90)	0.421

Abbreviations: CI, confidence interval; CML, carboxyl methyl lysine; PICF, peri-implant crevicular fluid; IFN, interferon; IL, interleukin; LDH, lactate dehydrogenase; MPO, Myeloperoxidase; TLR, Toll-like receptor; TNF, tumor necrosis factor.

assessed with the Wilcoxon rank test for two-group testing and the Kruskal-Wallis test for multigroup testing. Nonmetric multidimensional scaling analyses revealed clustering using Bray-Curtis's similarities or Jaccard's similarities[41] to evaluate beta diversity. Pairwise Wilcoxon rank sum tests were used to establish P-values. Pairwise Permanova test and Adonis test were used to test when there were significant beta diversity differences among different microbial communities.

For biomarker change from baseline, statistical analysis tested change from baseline vs. 0 with a two-sided paired-difference t-test.

Results

This study comprised 24 participants: 19 with mucositis (mean age, 46 years; range, 18-68 years; percentage female, 89%) and five with no mucositis (mean age, 56 years; range, 35-70 years; percentage female, 80%). There were no tobacco users. See Table 1. All implants were fabricated from titanium.

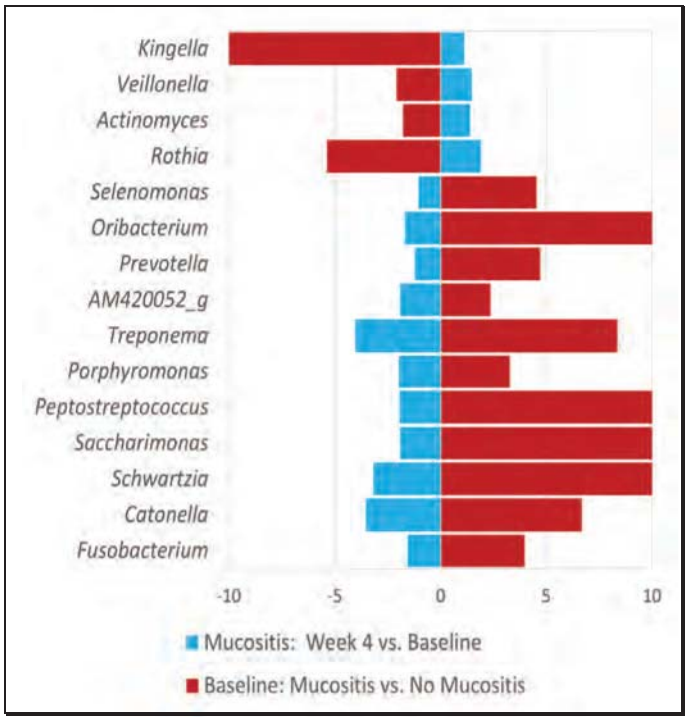

Fig. 1. The bar graph shows differential bacterial levels in mucositis vs. no mucositis groups at baseline (red) and in the mucositis group at week 4 vs. baseline (blue).

Clinical assessments - After 4 weeks of SnF_2 dentifrice use, all participants with mucositis exhibited a reduction in MGI and GBI scores versus baseline, with a 63.4% mean MGI reduction and a 79.31% mean GBI reduction ($P< 0.001$ for both). Subjects with mucositis also demonstrated a 72.45% reduction in mean number of bleeding sites ($P< 0.001$) relative to baseline (Table 2).

Biomarkers - After 4 weeks of SnF_2 dentifrice use, markers of bacterial insult and plaque virulence (TLR2 and TLR4 activity and endotoxins) were significantly reduced in plaque ($P< 0.001$ for all). Likewise, markers of oxidative stress (CML and LDH activity, $P\leq 0.0093$) and inflammation (IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-12p70, IL-13, and MPO; $P\leq 0.0401$) were significantly reduced in the PICF. IL-8 and TNF showed a decreasing trend in PICF (Table 3).

Microbiome - The study groups exhibited markedly different microbiome profiles at baseline. The mucositis group, in comparison with the no mucositis group, exhibited higher relative abundance of *Fusobacterium*, *Porphyromonas*, *Trepo-*

nema, and *Prevotella* and a lower relative abundance of *Rothia* and *Actinomyces*. After 4 weeks of SnF_2 dentifrice use by participants in the mucositis group, the relative abundance of these genera showed a trend toward the no mucositis microbiome profile, with a relative reduction in *Fusobacterium*, *Porphyromonas*, *Treponema*, and *Prevotella* and a relative increase in *Rothia* and *Actinomyces* (Fig. 1). Correlation analysis demonstrated that MGI, GBI, and the number of bleeding sites (BLD) were each significantly positively ($P \leq 0.05$) associated with host TLR2, TLR4, the total microbial endotoxins, and relative abundance of *Fusobacterium* and *Alloprevotella*, while negatively associated with relative abundance of *Rothia*. Many of the genera enriched in the mucositis participants' baseline microbiome profile such as *Porphyromonas*, *Tremponema*, *Catonella*, *Selenomonas*, and *Prevotella* also showed significant positive correlation with GBI and BLD. The TLR2 activity was positively associated with the relative abundance of *Fusobacterium*, *Prevotella* and *Selenomonas*, and the endotoxin level was positively associated with the relative abundance of *Fusobacterium*, *Prevotella*, *Porphyromonas* and *Selenomonas*, while negatively associated with the relative abundance of *Rothia* (Fig. 2).

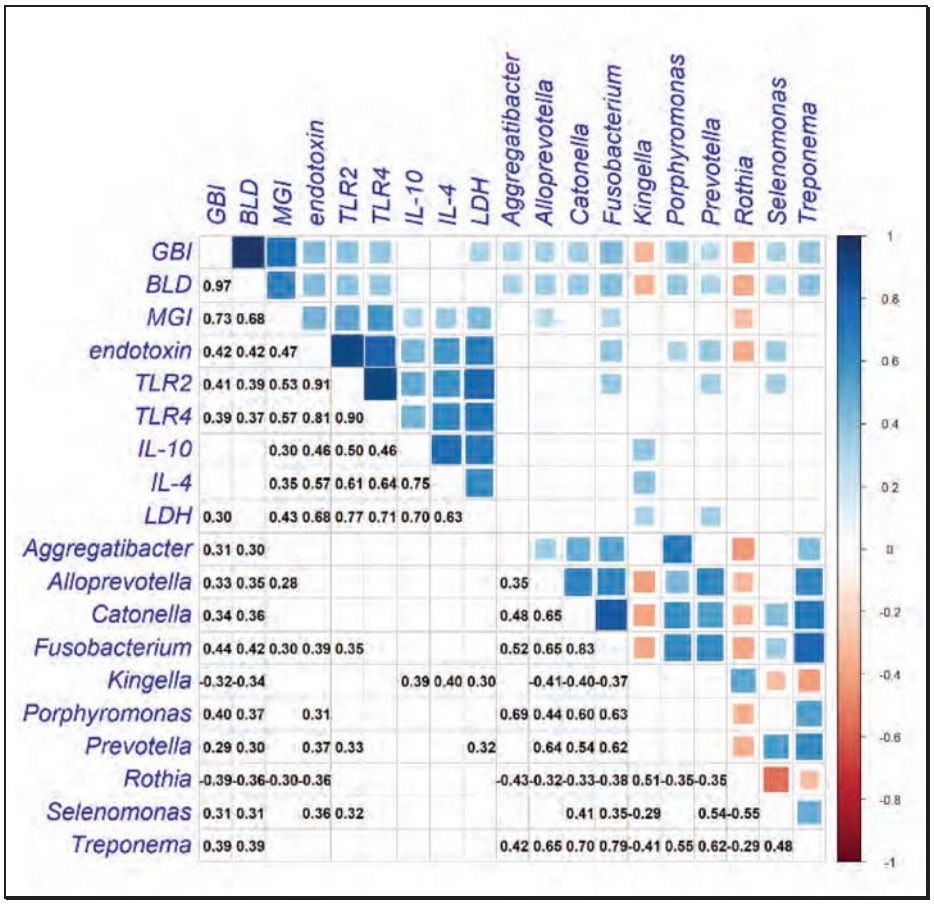

Fig. 2. Changes in clinical measurements (GBI: Gingival Bleeding Index, BLD: Number of Bleeding Sites, MGI: Modified Gingival Index) with biomarkers and correlation to oral microbiome. The correlation comparisons' directionality and magnitude are indicated by the color and size of the square (statistically significant correlations only, $P \leq 0.05$). Blue color indicates a positive correlation and red color indicates a negative correlation. Spearman correlation coefficient is indicated in the lower half of the graph.

Alpha diversity measured by the Shannon index in the mucositis group was higher than in the no-mucositis group (Fig. 3a, P= 0.039). Use of SnF_2 toothpaste for 4 weeks resulted in a reduction in Shannon diversity (Fig. 3a, P= 0.044). There were more observed microbial species in the mucositis samples (Fig. 3b, P= 0.033), but the SnF_2 toothpaste treatment did not reduce the species observed in subgingival plaque samples (Fig. 3b, P= 0.693). Beta diversity analysis with Bray Curtis dissimilarity measurement (Figs. 3c, 3d, 3e) revealed that the mucositis samples were significantly different from the no-mucositis samples (Pairwise Permanova test Mucositis Baseline vs. no-Mucositis Baseline, P= 0.004, adjusted P= 0.012). SnF_2 toothpaste treatment significantly shifted the microbial community with significant beta diversity differences before and after treatment (Fig. 3c, Pairwise Permanova test before and after SnF_2 toothpaste usage for Mucositis group: P= 0.039, adjusted P= 0.058). The diversity shift induced by treatment moved the microbiota toward the no-mucositis direction (Fig. 3d).

Simulated pathway and process analysis - Disease status (mucositis vs. no mucositis) was associated with significant differences of over two-fold ($P \leq 0.05$, Kruskal Wallis H test) in 1061 KEGG orthologs, 88% of which shifted toward the "no mucositis" profile after 4 weeks of SnF_2 dentifrice usage. Genes that were associated with mucositis state and later down-regulated following SnF_2 treatment included those for bacterial chemotaxis, endotoxin biosynthesis, flagellar assembly, toxins, proteases (e.g., gingipain), peptidases, and oxidative status modulators.

Safety - No adverse events were reported or recorded in this trial.

Discussion

A dentifrice containing SnF_2 significantly reduced multiple clinical signs of mucositis (MGI, GBI, and number of bleeding sites) in adults with peri-implant mucositis. This was accompanied by a significant reduction of endotoxins, an indicator of plaque virulence, along with a concomitant reduction in TLR2 and TLR4 activity indicating that the nuclear factor-κB (NF-κB) inflammatory pathway was inhibited. We also observed a reduction in oxidative stress markers (namely CML and MPO) and LDH, a cytosolic enzyme released from cell due to cellular injury. The reduction of LDH after SnF_2 treatment indicated improvement in gum health around the implant, consistent with the clinical observation. The reduction in many of the cytokines was also an indication of reduced inflammation. We also observed a reduction in IL-10 and IL-4 after treatment with SnF_2. Even though IL-10 and IL-4 have been considered as an anti-inflammatory cytokine, some previous studies[42,43] have shown elevated levels in saliva of peri-implant disease subjects compared to healthy subjects. Elevated levels of IL-1β and IL-10 have also been observed in peripheral blood mononuclear

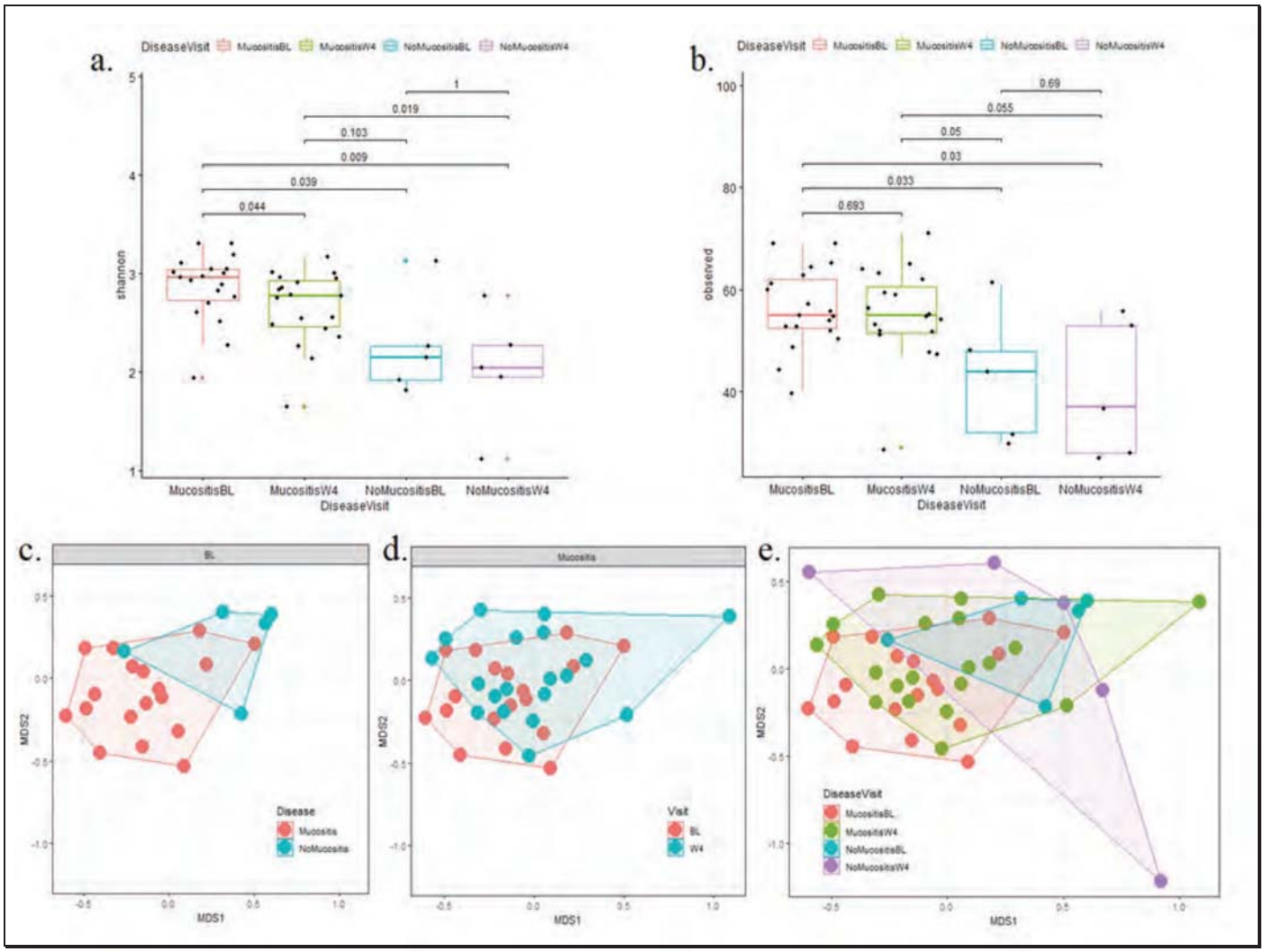

Fig. 3. Oral Microbiota Diversity analysis comparing 4 groups: Mucositis at Baseline (MucositisBL), Mucositis after 4 weeks of treatment (MucositisW4), No Mucositis at Baseline (NoMucositisBL) and No Mucositis after 4 weeks treatment (NoMucositisW4). (a) Average Shannon diversity measurement at baseline and after 4 weeks of treatment. Each box represents the Shannon diversity measurement from one group, and the line within each box represents the median. P-values of Wilcoxon tests for each pair of 2 groups are listed on the graph. (b) Average observed genus number at Baseline and after 4 weeks of treatment. Each box represents the Shannon diversity measurement from one group, and the line within each box represents the median. P-values of Wilcoxon tests for each pair of 2 groups are listed on the graph. (c) Beta diversity by Bray Curtis Distance: Multi-Dimensional Scaling analysis to evaluate sample differences among the mucositis and no mucositis groups at baseline (red dots: mucositis; green dots: no mucositis). Each data point represents an entire swab sample comprising many organisms. Each oral microbial community is plotted against all other communities. (d). Beta diversity by Bray Curtis Distance: Multi-Dimensional Scaling analysis to evaluate mucositis sample differences before and after treatment (red dots: Baseline; green dots: after 4 weeks treatment). Notice after 4 weeks of treatment, the microbial community shift towards the no mucositis group. (e) Beta diversity by Bray Curtis Distance: Multi-Dimensional Scaling analysis to evaluate both mucositis and no mucositis sample differences before and after treatment.

leukocytes in subjects with peri-implant mucositis compared to healthy subjects.[44] It was thought that IL-10 inhibits cytokine synthesis, but recent data suggests it has an immuno-regulatory role and certain conditions can result in dysregulated expression of IL-10.[45,46] Signs of mucositis and the abundance of pathogenic bacteria genera were positively correlated with biomarkers of bacterial insult. Endotoxins, a biomarker of bacterial insult, were negatively correlated with the abundance of Rothia, a genus commonly associated with a healthy oral microbiome.[47] Finally, simulated pathway and process analysis showed a shift from the mucositis profile toward the profile of the no mucositis group after 4 weeks of SnF_2 dentifrice usage.

It has long been established that dysbiotic plaque causes gingivitis,[31] and it is now considered as the cause of peri-implant mucositis as well.[3,4] In the case of gingivitis, SnF_2 dentifrice usage has been shown to reduce clinical evidence of inflammation and bleeding,[29] and this efficacy is attributable to the SnF_2-dependent reduction in plaque toxicity[26,27] and reduction in plaque regrowth after brushing.[28] Not only is plaque regrowth inhibited by SnF_2 dentifrice, but, among the bacteria that remain, the relative proportions of genera associated with oral health are significantly increased,[33] an effect that has been attributed to the selective binding of the Sn^{2+} ions to pathogenic bacteria.[34] SnF_2 dentifrice has also been shown to reduce endotoxins and mitigate harmful oxidant products both in saliva and gingival tissue.[32]

These health-promoting characteristics of SnF_2 dentifrices could reasonably be expected to benefit patients with peri-implant mucositis and thus warranted investigation. While positive outcomes for implant patients have been shown with home care regimens involving electric toothbrushes,[48,49] oral irrigators[50] and interdental devices,[51] to our knowledge, this is

the first published paper reporting on effects of SnF_2 dentifrice for mitigation of peri-implant mucositis. Given that good oral hygiene is essential to reduce and prevent peri-implant mucositis, peri-implantitis, and implant loss,[3-5] the results of this study point to SnF_2 dentifrice as a practical, cost-effective, and easily accessible way to maintain a healthy implant. Recommending this at-home, preventive strategy aligns with recent guidance for dental professionals to rely more on prevention and less on intervention.[52] The emphasis on primary prevention is particularly important in light of a recent survey among periodontal patients indicating about half were unaware that an implant could end in failure and only 28% had heard of peri-implantitis.[53]

The strengths of this study include its use of established experimental methods and its comprehensive analysis, which encompassed clinical signs, biomarker analysis, and microbiome profiling. Additionally, because this study tested a dentifrice already proven to maintain gingival health,[29,54] these results add to the body of evidence so dental professionals can confidently recommend this SnF_2 dentifrice for the maintenance of both overall gingival health and implant health. These results could be expanded by conducting a randomized, controlled study and by extending the treatment period beyond 4 weeks to assess longer-term effects. Additionally, since the antigingivitis efficacy of SnF_2 dentifrices has been shown to depend on formulation,[55] the comparison of different formulations with respect to anti-mucositis efficacy would be informative.

a. Oraflow R Inc. New York, NY, USA.
b. Thermo Fisher Scientific, Waltham, MA, USA.
c. MilliporeSigma, Burlington, MA, USA.
d. Sciex, Framingham, MA, USA.
e. Meso Scale Diagnostics, Rockville, MD, USA.
f. EzBiome, Gaithersburg, MD, USA.
g. The Procter & Gamble Company, Cincinnati, OH, USA.
h. The R Foundation for Statistical Computing, Vienna, Austria.

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