The efficacy of stabilized chlorine dioxide rinse as a chemical adjuvant for treatment of Peri-Implant Mucositis

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Final Report

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Product:

Stabilized Chlorine Dioxide Oral Rinse
ClōSYS® Ultra Sensitive Rinse
0.1% (w/v) Stabilized Chlorine Dioxide

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The efficacy of stabilized chlorine dioxide rinse as a chemical adjuvant for treatment of Peri-Implant Mucositis.

[1] Introduction

The use of dental implants has revolutionized the treatment of partially and fully edentulous patients today. Implants have become a treatment approach for managing a broad range of clinical dilemmas due to their high level of predictability and their ability to be used for a wide variety of treatment options. While in many cases dental implants have been reported to achieve long-term success, they are not immune from complications associated with improper treatment planning, surgical and prosthetic execution, material failure, and maintenance. Included in the latter are the biologic complications of peri-implant mucositis and peri-implantitis, inflammatory conditions in the soft and hard tissues at dental implants. Peri-implant mucositis is characterized by inflammation in the mucosa around the implant without signs of bone loss. If bone loss also occurs, the condition is designated as peri-implantitis. Studies reported 43% and 22% prevalence of peri-implant mucositis and peri-implantitis, respectively. Peri-implant mucositis is a reversible condition, but if left untreated can progress to peri-implantitis. Besides mechanical treatment, other therapies have been used for treatment of peri-implant mucositis, namely: 1) triclosan-based dentifrice; 2) abrasive air blasting with sodium carbonate and resin curets; 3) mouth rinses with 0.2% chlorhexidine and 1% gel; 4) 0.5% chlorhexidine gel; and 5) irrigation with 0.12% chlorhexidine solution, as well as use of systemic antibiotics.

Stabilized chlorine dioxide exhibits antimicrobial properties against oral bacteria. Clinical and laboratory evidence suggest that stabilized chlorine dioxide oral rinse reduces the number of bacteria in the mouth, essentially eliminates oral malodor, reduces the signs of gum disease, and has bactericidal properties comparable to other products with additional consumer acceptability relating to its non-alcoholic and non-staining features. Thus, the purpose of this study was to analyze efficacy of stabilized chlorine dioxide mouth rinse as a chemical adjuvant for treatment of peri-implant mucositis in a non-surgical treatment protocol with a 3-month follow-up.

[2] Objective

A full understanding of etiology and diagnosis of peri-implant diseases is crucial for finding effective treatments for these diseases that are more widely accessible to dentists. Several treatment protocols for peri-implant diseases have been proposed, but no gold standard has been established to date. Thus, the purpose of this study was to analyze efficacy of stabilized chlorine dioxide as a chemical adjuvant for treatment of peri-implant mucositis in a non-surgical treatment protocol with a 3-month follow-up. A collection swab was performed for microbiome analysis of the mouth on three visits.

[3] Study Design

Fifty-four individuals with peri-implant mucositis were identified to participate in this study and randomized into two groups to analyze clinical parameters and results of this study: 1) test group (stabilized chlorine dioxide rinse) and 2) control group (placebo). Each group was associated with periodontal basic therapy.

Treatment Protocol
After inclusion of the patients, medical history and initial examination were performed, patients were randomly divided into the following two groups: 1) test (stabilized chlorine dioxide rinse associated with periodontal basic therapy); and 2) control (placebo associated with periodontal basic therapy).

Periodontal basic therapy consisted of oral hygiene instruction, motivation, retentive factor removal, and an adaptation of the protocol of full-mouth scaling and root planing (stabilized chlorine dioxide mouthwash will be used). Plastic currets were used to instrument the implants, and metal currets to instrument teeth. Immediately after instrumentation, chemical solutions of chlorine dioxide or placebo were dispensed to the subjects.

The subjects followed their normal oral-hygiene procedures with the addition of using the study rinse toothbrush and toothpaste provided to them.

Overview of the Sample Collection Protocol for Microbiome Analysis

Samples for microbiome analysis were collected at baseline (Visit 1), Day 14 (Visit 2), and Day 90 (Visit 4). Before collecting samples, each patient was asked to rinse with plain water prior and rest for 5 minutes. Sample collection was performed by gently wiping the buccal and tongue mucosa separately 10 times with three different cotton swabs. Samples were allowed to air dry at room temperature and individually placed in labeled containers with the patient's unique sequential subject identification number and day of collection. These samples were stored in a secure location at -20°C until utilized for microbiome analyses.

[4] Study Design Table

Protocol Study Design and Schedule of Assessments

Evaluation	**Screening** (0-2wks to Baseline)	**Baseline (Visit 1)** Day 0	**Visit 2** Day 14 (± 3 d)	**Visit 3** Day 45 (± 7 d)	**Visit 4** Day 90 (± 7 d)
Informed Consent	x				
Inclusion / Exclusion	x	x			
Demographics	x				
Pregnancy Test		x			x
Medical History / Update	x	x	x		x
Medications / Update	x	x	x		x
Periodontal History	x		x		x
Oral Examination	x	x	x		x
Collect Specimen w Swab		X	X		X
Modified Gingival Index / MGI		x	x		x
Bleeding On Probing / BOP	x				x
Probing depth	x	x			x
Turesky modification Plaque Index		x	x		x
Scaling	x				
Eligibility	x				
X-rays		x			x
Enrollment / Randomization		x			
Dispense oral hygiene kit / Diary		x			
Schedule next appt	x	x	x		
Collect residual oral hygiene kit with Diary and distribute oral hygiene kit with Diary for next phase			X	x	
Collect residual oral hygiene kit / Diary					x

[5] Protocol Synopsis

1. Study Title: The Efficacy and Safety of stabilized chlorine dioxide mouth rinse in Therapy of Peri-implant mucositis

2. Study Design: Single Center, randomized, double blind masking, parallel, two-arm clinical study.

3. Objective: The purpose of this study was to analyze efficacy of stabilized chlorine dioxide as a chemical adjuvant for treatment of peri-implant mucositis in a non-surgical treatment protocol with a 3-month follow-up.

4. Study Treatments: 1) Stabilized chlorine dioxide (associated with periodontal basic therapy); and 2) control (placebo associated with periodontal basic therapy).

5. Number of Patients: A total of 54 patients in one clinical site at Stony Brook were randomized, 27 patients received 1) Stabilized chlorine dioxide (associated with periodontal basic therapy); and 27 patients received 2) control (placebo associated with periodontal basic therapy).

6. Study Duration and Visits: The study duration was (3 months) and was comprised of a total of 4 visits: Screening and hygienic phase therapy (weeks -2 to -1), Baseline (Visit 1, Day 0), Visit 2 (14 days), Visit 3 (45 days), Visit 4 (90 days).

7. Subjects received an Oral Hygiene Kit at Visit 1. The Modified Gingival Index (MGI), Bleeding on Probing (BOP) and Plaque Index (PI), Pocket depth and radiographs were measured, and all oral tissues examined (baseline examination). Subjects returned for Visit 2 in 14 days ± 3 days to: 1) assess and record changes in indices and oral health and any adverse conditions. Visit 3 (45 days ± 7) was a compliance visit for study drug utilization. Visit 4 (90 days ± 7) was a repeat of Visit 1. Samples for microbiome analysis were collected at baseline (Visit 1), Day 14 (Visit 2), and Day 90 (Visit 4).

8. All parameters for clinical exam were calibrated between the examiners involved with the study.

[6] Study Materials (Oral Hygiene Kit)

STUDY DRUG

Both the active and placebo drugs were provided in liquid form, intended for oral administration. Sample products were labeled by the manufacturer and shipped directly to the site.

ADMINISTRATION

After tooth brushing in the morning and evening, the study product was administered orally using a cup that measures 15 mL of oral rinse, swishing in the oral cavity for 30 seconds and expectorating.

[7] Subjects

Subject recruitment occurred at the discretion of the Principal Investigator. After being recruited, potential subjects were informed of the purpose of the study and guided through HIPAA documentation and the informed consent process. Upon their understanding of the study, HIPAA regulations, and their agreement with the terms of the informed consent, subjects indicated so by signing the forms. The eligibility of potential subjects was determined through a series of screening interviews and an oral examination. Following screening, those potential subjects who retained eligibility were enrolled in the study at the discretion of the Principal Investigator. Approximately 75 subjects were screened to provide 54 enrollees. Once these subjects were randomized, study enrollment was complete.

Number of subjects: 54 subjects

Age: 18 years to 80 years

Gender: Both male and female subjects entered this study.

Remaining Natural Teeth: Minimum of 20

[8] Subject Eligibility Criteria

Each subject was enrolled into the study based upon an initial interview and screening examination certifying the following conditions:

INCLUSION CRITERIA

Inclusion criteria included systemically healthy, partially edentulous patients rehabilitated with functional dental implants and prostheses for at least 1 year, at the Department of Periodontics Stony Brook University. All patients were selected from Stony Brook Dental Clinic and were detected with different peri-implant diagnoses.

Patients included had:

1. diagnosis of peri-implant mucositis,
2. at least at one implant;
3. minimum of 20 natural teeth
4. probing depth (PD) ≤5mm;
5. BOP (bleeding on probing);
6. No radiographic evidence of bone loss beyond the first two threads of the implant.

EXCLUSION CRITERIA

1. Active Periodontitis or Peri-implantitis, which required definitive treatment.
2. Presence of oral local mechanical factors that could have (in the opinion of the PI) influenced the outcome of the study.
3. Presence of orthodontic appliances, or any removable appliances, that impinged on the tissues being assessed.
4. Presence of soft or hard tissue tumors of the oral cavity.
5. Patients treated with systemic antibiotic therapy or periodontal/mechanical/local delivery therapy within 12 weeks prior to study entry and throughout the study duration.
6. Patients chronically (i.e. two weeks or more) treated with non-steroidal anti-inflammatory drugs (NSAIDs) or any medications known to affect soft tissue condition (excluding treatment of Acetylsalicylic acid ≤ 100 mg/day).
7. Patients with uncontrolled diabetes, of any type, and/or patients with HbA1c test value >7.5% dated 3 months prior to the screening visit.
8. Patients who were receiving radiation therapy to the head and neck area and/or receiving immunosuppressive therapy.
9. The presence of any medical or psychiatric condition or any other condition that, in the opinion of the Investigator, could have affected the successful participation of the patient in the study.
10. Drug and alcohol abuse.
11. Patients who were participants in any other clinical study 30 days prior to the start of the study and throughout the study duration.
12. Subject was pregnant (based on pregnancy result) or lactating.
13. Subject was a smoker or had been a smoker within the past 6 months.
14. Any other condition that may have interfered with the study as judged by the PI.

ASSIGNMENT

Once a subject had been screened and qualified for study participation, that subject was enrolled and assigned the next available randomization number. The study randomization table was generated by a third-party statistician. This procedure was used to keep the Study Statistician blinded to subject treatments prior to database lock.

WITHDRAWAL

Subjects enrolled in the study were able to elect to withdraw at any time for any reason. Additionally, subjects were able to have been withdrawn from the study at the request of the Principal Investigator for the following reasons:

1. An adverse event required discontinuation of the study rinse in the judgment of the Principal Investigator or designee
2. Subject refused or failed to comply with the study protocol

3. There was a protocol violation(s) or deviation(s) that compromised the use of the subject's data

All patients withdrawn from the study by request or by the Principal Investigator were to be seen, if they consented, for a close-out evaluation. The reason for withdrawal would be recorded by the Principal Investigator or designee on the End of Study form. Patients who experienced complications and discontinued the study on their own were to be scheduled, if they consented, for an immediate follow-up/close-out examination to determine whether an adverse event was present, and if so, the causation of the adverse event was to be recorded. If treatment was needed for any adverse event causing withdrawal, the subject would be monitored until there was a return to normal conditions. (Every effort would be made to follow up with subjects who withdrew from the study.)

Test Product: ClōSYS® Ultra Sensitive Rinse

Placebo: Oral rinse comprising same ingredients as in test product except for stabilized chlorine dioxide.

Materials:

Sponsor shipped prelabelled 'Oral Rinse A' and 'Oral Rinse X' products to the Test Site. Both products were in 16 oz. size bottle and identical in appearance.

Each subject received the following supplies at Baseline (Visit 1) i.e. supplies for 0-14 days:

a. 1 bottle (16oz) of either Oral Rinse Aor Oral Rinse X))
b. 35 measuring cups
c. 1 tube of Crest Cavity Protection Toothpaste, Regular (8.2oz) and
d. 1 ClōSYS toothbrush

Each subject received the following supplies at Visit 2 i.e. supplies for 15-45 days:

a. 2 bottles (16oz) of either Oral Rinse A or Oral Rinse X)
b. 70 measuring cups
c. 2 tube of Crest Cavity Protection Toothpaste, Regular (8.2oz) and
d. 3 ClōSYS toothbrush

Each subject received the following supplies at i.e. Visit 3 Compliance supplies for 45-90 days:

a. 3 bottles (16oz) of either Oral Rinse A or Oral Rinse X
b. 105 measuring cups
c. 2 tube of Crest Cavity Protection Toothpaste, Regular (8.2oz) and
d. 3 ClōSYS toothbrush

Rowpar shipped the following materials to the Study Site (10% extra quantities were included for handling unforeseen situations):

a. 165 bottles of 16-oz Oral Rinse A.

b. 165 bottles of 16-oz Oral Rinse X.
c. 116 packs of 100 measuring cups.
d. 165 tubes of Crest Cavity Protection Toothpaste, Regular (8.2oz)
e. 220 ClōSYS toothbrushes.
f. 5 boxes of 28 Ziploc bags-1 Gallon capacity

The study site coordinator arranged to prepare Ziploc bag packets containing required supplies for distribution to each Subject.

[9] Study Procedures

SCREENING

Review of the results of the Informed Consent, Screening Interview and Screening Oral Examination, the Principal Investigator or designee determined whether or not a subject was enrolled in the study. The following procedures were conducted:

1. Informed Consent:

2. Screening Interview: The subject was asked inclusion and exclusion questions, demographics, and date of last professional cleaning. The following was also reviewed as reported by the subject:

    a. Medical History and Medications

    b. Periodontal treatment history

3. Oral Examination:

    a. Extra- and Intra- oral examination

    b. BOP oral examination and probing depth

4. Eligibility Determination:

    a. If the subject qualified, an appointment date was set for the Baseline Examination (Visit 1) to occur within 14 days of the screening examination or could be conducted at the same time as the screening visit.

VISIT 1 - BASELINE EXAMINATION – DAY 0

This visit took place within two weeks of the initial screening visit or could be conducted at the same time as the screening visit.

The following procedures were conducted:

1. Reviewed any new medication(s) or medical history on respective documents.
2. Rinsed mouth with water and collected samples for microbiome analysis by use of a cotton swab on the inside of the cheek and tongue mucosa.
3. Performed pregnancy testing on females of childbearing age.
4. Evaluated oral mucosal irritation or pathology.
5. Performed a Modified Gingival Index (MGI) grading on all natural teeth and implants in the dentition.
6. Performed a Turesky modification of the Quigley-Hein Plaque Index (PI) grading on all natural teeth and implants in the dentition.
7. Assessed bleeding on probing (BOP) on all natural teeth and implants in the dentition.
8. Verified data capture.
9. X-rays
10. Full mouth Scaling
11. Randomization and dispensation of the Oral Hygiene Kit and Oral Hygiene Diary.
12. The subjects then followed their normal oral-hygiene procedures with the addition of using the study rinse, toothbrush, and toothpaste provided to them.
13. Provided diary for recording use of product rinse or placebo.
14. Provided supplies for 14 days (day 1 to day 14 of the study). Weighed and recorded the weight of test rinse or placebo, as applicable, being provided.
15. Instructed Subject to bring remaining oral rinse or placebo bottle
16. Scheduled following appointments for Visit 2 (2 weeks after base line) and Visit 3 (3 months) and noted in their study instructions and diary. Subjects were to be seen within plus or minus 7 days.

VISIT 2 - INTERIM EXAMINATION - DAY 14

This examination took place within 14 calendar days after Baseline Examination (Visit 1) ± 3 days. The following procedures were conducted:

1. Reviewed any change in medical and dental history
2. Rinsed mouth with water and collected samples for microbiome analysis by using a cotton swab on the inside of the cheek and tongue mucosa.
3. Evaluated extra- and intra- oral areas for any mucosal irritation or pathology.
4. Performed a Modified Gingival Index (MGI) grading on all natural teeth and implants in the dentition.
5. Performed a Turesky modification of the Quigley-Hein Plaque Index (PI) grading on all natural teeth and implants in the dentition.
6. Weighed and recorded remaining test rinse or placebo as applicable.
7. Checked record for use of test rinse or placebo in the diary.
8. Provided diary for recording use of product rinse or placebo.
9. Provided supplies for 30 days (day 15 to day 45 of the study). Weighed and recorded the weight of test rinse or placebo, as applicable, being provided.
10. Confirmed appointment date scheduled for the Compliance Visit (Visit 3). The subjects were given a reminder call 2 - 3 days prior to their scheduled appointment.

VISIT 3 – COMPLIANCE VISIT – DAY 45

This examination took place within 45 calendar days after Baseline Examination (Visit 1) ± 7 days. The following procedures were conducted:

1. Weighed and recorded remaining test rinse or placebo as applicable.
2. Checked record for use of test rinse or placebo in the diary.
3. Provided diary for recording use of product rinse or placebo.
4. Provided supplies for remaining 45 days (day 46 to day 90 of the study). Weighed and recorded the weight of test rinse or placebo, as applicable, being provided.
5. Confirmed appointment date scheduled for the Final Examination Visit (Visit 4). The subjects were given a reminder call 2 - 3 days prior to their scheduled appointment.

VISIT 4 - FINAL EXAMINATION – DAY 90

This examination took place within 90 days following the Baseline Examination (Visit 1) ± 7 days. The following procedures were conducted:

1. Collected the subject's diary and unused products at check-in. All partially used products were assessed for volume remaining and recorded. Used products were retained until after a monitoring visit, and then returned to the Sponsor.
2. Performed pregnancy testing on females of childbearing age.
3. Rinsed mouth with water and collected samples for microbiome analysis by use of a cotton swab on the inside of the cheek and tongue mucosa.
4. Asked the subject to report any changes in medical or dental health since the last visit.
5. Evaluated extra- and intra- oral areas for any mucosal irritation or pathology.
6. Performed a Modified Gingival Index (MGI) grading on all natural teeth and implants in the dentition.
7. Performed a Turesky modification of the Quigley-Hein Index (PI) grading on all natural teeth and implants in the dentition.
8. Assessed bleeding on probing (BOP) and probing depth on all natural teeth and implants in the dentition.
9. X-rays
10. Weighed and recorded remaining test rinse or placebo as applicable.
11. Checked record for use of test rinse or placebo in the diary.

[10] Study Timeline

Using December 2018 as a calendar starting point, the entire month would provide 20 days for recruiting. As the screening, cleaning and baseline visit can be all on the same day we were considering that subjects could begin Study Visit 1 between January 2019 and December 2019. The final study visits was conducted in March 2020. A final report concluded by December 2020.

[11] Statistical Evaluation

The primary objective of this study was to analyze efficacy of stabilized chlorine dioxide as a chemical adjuvant for treatment of peri-implant mucositis in a non-surgical treatment protocol with a 3-month follow-up.

The primary objective was determined by an examination of the following data:

a) Gingival inflammation (MGI)

Secondary analyses were the comparison of data with respect to product effectiveness:

a) Reduction in bleeding on probing (BOP) and Turesky modification of the Quigley-Hein Plaque Index (PI) grading on all natural teeth in the dentition

The primary statistical analytic framework was the repeated measures analysis of variance on three primary outcomes: probing depth, gingival index, and plaque. The primary hypothesis tested was the treatment by time interaction, i.e., did one treatment work better during the observations of these three outcomes? In other words, not just the total change, but also the rate of that change might differ between treatments. The proposed analysis was based on data that were generally normally distributed and similarly. If not, data transformations or link functions were used.

Outcomes

Raw data were collected from examinations of each tooth with regard to probing depth, gingival index, and plaque. Data were collected over three time periods, recorded into patient files, and transferred to Excel Workbooks, with one Workbook per patient. Each Workbook contained three tabs, one for each visit.

The raw data were combined as two levels of indexes for the three measures. The first outcome was a global score index of probing depth, gingival index, and plaque measurement. The global index was derived from individual tooth scores for each measure divided by the number of teeth measured. The second index derived from the same raw data described the three measures in terms of average scores for implant and non-implant teeth.

For the second analysis, implanted tooth data were separated from normal teeth data within each patient. Implant scores for each patient were averaged for probing depth, gingival index, and plaque scores. If a patient had one implant, each score was identical to the single measurement. With more than one implant, a single implant score for each patient was derived from averaging each of the three measures over the three observation periods. Likewise for each patient, the non-implanted teeth measures were averaged for each of the three times of assessment.

Individual patient data of the averages of the implant and non-implant teeth were calculated for each time period and transferred to a master data sheet.

Data Management and Statistical Analysis

The patients' data on the three measures were recorded within the Excel Workbooks under three tabs representing the first, second, and third clinical observation periods. So each tab recorded three measures. The first and last tabs recorded probing depth, gingival index, and plaque. The second visit included only gingival index and plaque. Each patient had an individual Excel Workbook. Data were sorted into analysis format through two Excel macros that created a single master data for global scores and a single one for implant and non-implant scores. Data files were saved in comma separated value (csv) format in both wide and long formats for R and SPSS data management, inspection, cleaning, and analyses.

Since many individual variable distributions varied substantially from normal, a gamma log-link function was applied to reduce bias in most statistical tests.

Label Key and Lot #s for Peri-Implant Mucositis Study with Stony Brook University

Rowpar Pharmaceuticals, Inc., released the test material label codes after completion of the clinical study and statistical analysis of the data. The products were labelled as follows:

Study Number: SBU-RPR-Peri-Implant Mucositis-2018

Oral Rinse A: ClōSYS Placebo Oral Rinse, Lot #: 2-80516

Oral Rinse X: ClōSYS® Ultra Sensitive (Unflavored) Oral Rinse, Lot #: 8122A 14-80430

Results

Patients

Fifty-two percent (29/56) of patients in both groups had only one implant. Patients in the control group (Group A) had a total of 47 implants: 11 patients had just one implant, 13 had 2, 2 had 3, and 1 had 4 implants. In the test group (Group X), there were 42 total implants in 26 patients: 18 had 1 implant, 9 had 2, and 2 had 3 implants (see Figure 1). Table 1 gives average values (and standard error of the mean) of Gingivitis Index, Plaque score, and Probing depth at baseline.

Figure 1. Bar chart of numbers of implants per person in control group (Group A) and test group (Group X).

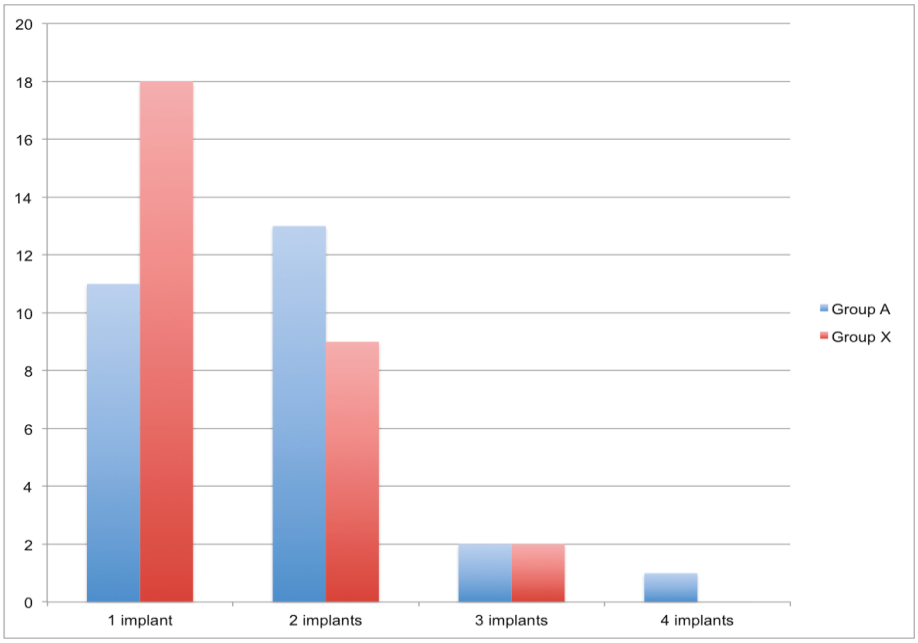


Table 1. Patient dental measures at baseline.

		n of patients	n of implants	Modified Gingivitis Index (MGI) (sem)	Plaque score (PS) (sem)	Probing Depth mean (sem)
Control Group (Group A)	implants	27	47	2.06 (0.048)	2.77 (0.115)	3.40 (0.109)

	non-implanted teeth			1.87 (0.039)	2.32 (0.101)	2.72 (0.099)
Test Group (Group X)	implants	26	39	2.82 (0.049)	2.84 (0.057)	2.447 (0.080)
	non-implanted teeth			2.01 (0.028)	2.99 (0.078)	2.65 (0.079)

Global Score Analyses

From raw data of individual teeth, each patient received a global index of periodontal disease, gingival index, and plaque for each visit. The global index for each of these measurements was found by dividing the sums of patient probing depth, gingival index, and plaque findings collected for each tooth by the number of teeth assessed. Over the three assessment periods, global scores of probing depth, gingival index, and plaque were collected for the first and last visits with probing depth scores not collected during on the second visit. These outcomes are measured as individual slopes of responses across the three observation visits. The slope represents the rate of change.

The overall view of the response of each measure to the treatment group is summarized in the following general linear model Table 2. Table 3 shows the cell statistics for the slopes of each condition and outcomes reported in Table 2.

Table 2. Master table of variance components attributable to treatment on individual rates of change on the three measures: probing depth, gingival index, and plaque.

Source	Variable	Type III SS	df	MS	F	Sig.
Group	Slope of probing depth, time 1 to 3	0.125	1	0.125	1.191	0.28
	Slope of gingivitis index, time 1 to 3	0.485	1	0.485	11.748	0.001
	Slope of plaque score, time 1 to 3	4.019	1	4.019	69.751	0.000
Error	Slope of probing depth, time 1 to 3	5.75	55	0.105		
	Slope of gingivitis index, time 1 to 3	2.27	55	0.041		
	Slope of plaque score, time 1 to 3	3.169	55	0.058		
Total	Slope of probing depth, time 1 to 3	6.519	57			

	Slope of gingivitis index, time 1 to 3	6.135	57
	Slope of plaque score, time 1 to 3	18.861	57
Corrected Total	Slope of probing depth, time 1 to 3	5.875	56
	Slope of gingivitis index, time 1 to 3	2.755	56
	Slope of plaque score, time 1 to 3	7.187	56
R Squared = .021 (Adjusted R Squared = .003)			
R Squared = .176 (Adjusted R Squared = .161)			
R Squared = .559 (Adjusted R Squared = .551)			

Table 3. Details of cell statistics for analysis described in Table 2.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Probing depth at time 1	57	2.04	4.63	2.71614	0.432843
Probing depth at time 3	57	0	4.42	2.50351	0.804056
Gingivitis index at time 1	57	1.41	2.23	1.97158	0.172065
Gingivitis index at time 2	57	0	2	1.71754	0.364095
Gingivitis index at time 3	57	0	1.92	1.48456	0.451688
Plaque score at time 1	57	1.47	3.54	2.67895	0.555475
Plaque score at time 2	57	0	3.11	2.19614	0.596444
Plaque score at time 3	57	0	2.92	1.77386	0.618124
Slope of probing depth, time 1 to 3	57	-1.47	0.015	-0.10632	0.323886
Slope of gingivitis index, time 1 to 3	57	-0.99	-0.03	-0.24351	0.221807
Slope of plaque score, time 1 to 3	57	-1.53	-0.045	-0.45254	0.358255
Valid N (listwise)	57				

Global Probing Depths (GPD)

Global probing depth (GPD) collected at the first visit did not differ significantly between groups; control group's (Group A) initial GPD was 2.76 (SEM = 0.092) interval, and 2.68 (0.071) for test group (Group X), a non-significant statistical difference (p = 0.48).

Neither did the GPD score differ between final visits of control group (Group A) (GPD = 2.64 (0.130) and test group (Group X) (GPDI = 2.37, (0.166), t = 1.27, df = 55, p = 0.28. Nonetheless, the GPD decreased significantly for control group (Group A), (p = 0.028; for X, p < 0.001) for both groups, but the group decreases were not significantly different from each other. Both mouthwashes reduced probing depth similarly.

The key signal of treatment effectiveness is a statistically significant treatment-by-time interaction, where improvement over time is greater for one treatment than another. Nearly parallel lines in Figure 2 and statistical testing indicated no appreciable and significant treatment-by-time interaction for GPD (See Figure 2, Figure 3).

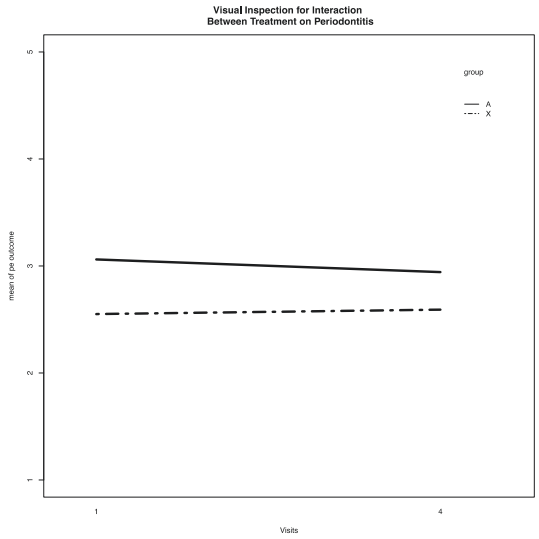


Figure 2. Interaction plot showing that group assignment did not interact with time for probing depth.

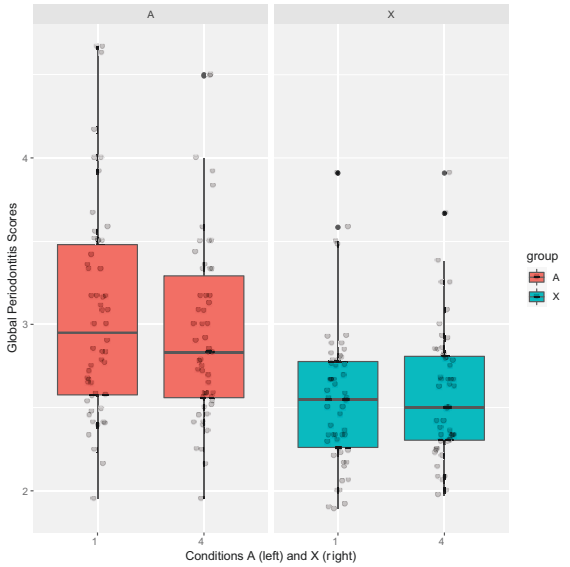


Figure 3. Boxplots of probing depth results from treatment by control group (Group A) and test group (Group X). Probing depth was not affected differentially by the mouthwash treatments. Both conditions reduced probing depth slightly but not statistically significant.

Global Plaque Score

Control group (Group A) and test group (Group X) differed significantly in global plaque score (GPS) at initial assessment, requiring an additional analysis of covariance to confirm that a significant time by treatment interaction was not an artifact from the initial difference in GPS (See interaction plot, Figure 4, detailed in Figure 5). The test group's (Group X) initial average GPS was 3.00 (SEM = 0.071) versus 2.35 (0.097) for the control group (Group A), a significant difference (t = -5.45, df = 50, p < 0.001). At assessment 3, the last observation, the test group's (Group X) GPS (1.57, SEM = 0.110) was significantly lower than that for the control group (Group A); GPS = 1.98 (0.110), t = 2.633, df = 55, p = 0.011.

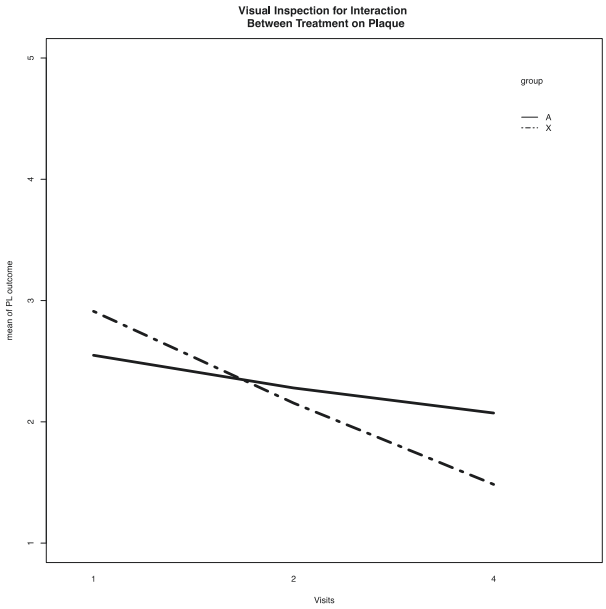


Figure 4. Interaction plot showing that group assignment interacted with time on the measure plaque.

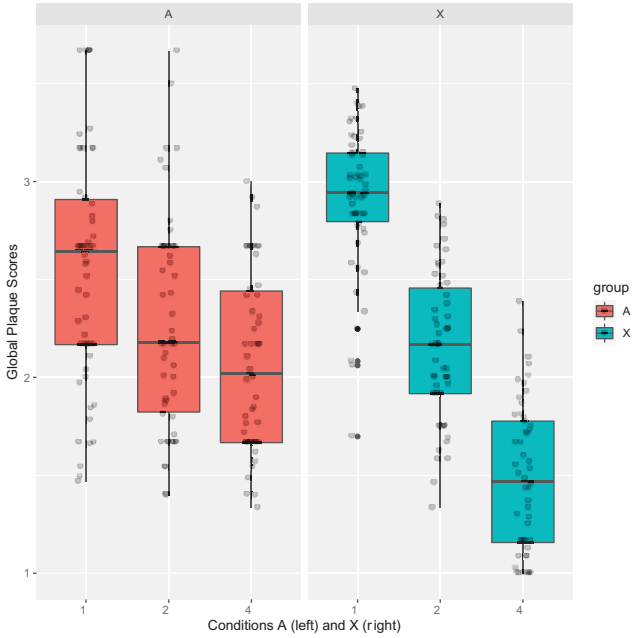


Figure 5. Boxplots of plaque measures comparing treatment control group (Group A) and test group (Group X) for GPS. Test group (Group X) started with higher levels of plaque. Plaque decreased more in the test group (Group X) than in the control group (Group A), though both decreased significantly.

An analysis of covariance (ANCOVA) with the first visit as covariate was used to statistically control for the difference in average plaque scores at intake. ANCOVA results confirmed that test group (Group X) produced a significantly lower GPS measures (ANCOVA $F = 34.82$, $df = 1$, $p < 0.0001$), even with significant differences in the initial scores.

To further explore and confirm control rinse (rinse A) and test rinse (rinse X) treatment effects on plaque measurement, from Time 1 to Time 3, both a Student's t-test and a Bayesian analysis of group means were employed. The Bayesian analysis supported the ANCOVA results.

Another t-test showed test group's (Group X) GPS absolute change and rate of change were significantly greater than for control group (Group A): -1.43 versus 0.365, respectively. This difference is considered a large effect size, Cohen's $d = 2.21$ ($t = 8.35$, $df = 1$, $p < 0.0001$). Thus, the changes and rates of change for GPS were greater for the test group (Group X) than the control group (Group A).

Global Gingival Index Score

The gingivitis index is based on the Gingival Index system,[2] in this case, the global gingival index is GGI. The GGI was also significantly higher for the test group (Group X) at the initial visit; for the test group (Group X) = 2.03 (0.025) and GGI for the control group (Group A) = 1.91 (0.035), (t = -2.89, df = 55, p = 0.006). Figure 6 illustrates a potential interaction between treatment and time of observation that required statistical consideration and control. Again, GGI for visit 1 was used as a covariate so as not to over-estimate treatment effects on GGI represented in a time-by-treatment interaction. The ANCOVA upheld a statistically significant (t = 2.12, df = 51, p = 0.039) between treatments. Boxplots of the GGI across visits is shown in Figure 7. The test group (Group X) reduction in GGI was 27.3% versus the control group's (Group A) reduction of 20.4%, also statistically significant difference by repeated measures analysis of variance (F = 8.141, df = 1, p = 0.0046).

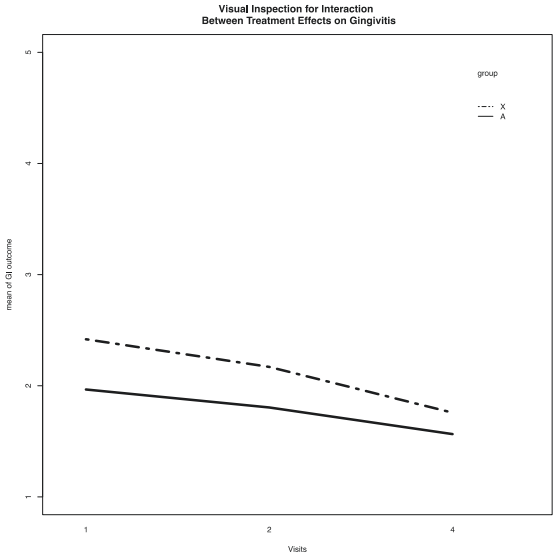


Figure 6. Interaction plot showing group assignment interacting with time on gingival index measures (GGI).

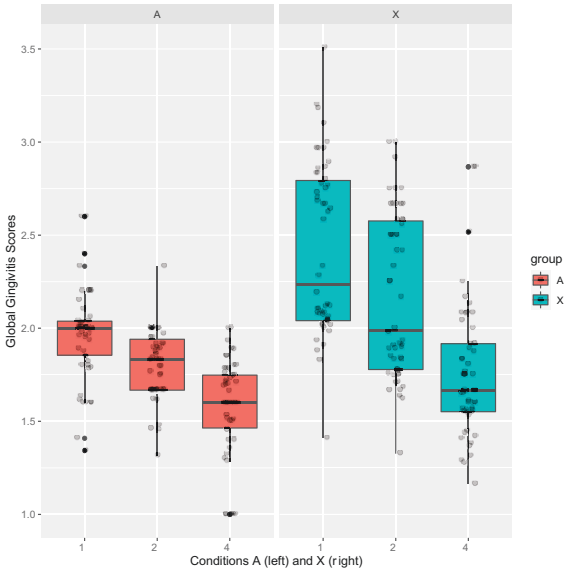


Figure 7. Boxplots of gingival index measures comparing treatment control group (Group A) and test group (Group X). Test group (Group X) started with higher gingival index levels. Gingival index decreased more and faster in test group (Group X) than in the control group (Group A), though both decreased significantly.

Differential Effects of Mouthwashes on Implants in Control Group (Group A) and Test Group (Group X)

Of particular interest was the specific effect that control mouthwash (rinse A) and test mouthwash (rinse X) might have on implant and non-implant sites across the three outcomes. Table 4 illustrates the overall study results of treatments and implants on the three outcomes. Probing depth was not different between treatments for implant and non-implant sites. Plaque and gingival indexes were improved significantly under test rinse Treatment (rinse X), with details described in Tables 5, 6, and 7 below. In addition, implant sites improved at a faster rate under test rinse Treatment (rinse X) conditions for both gingivitis and plaque.

Table 4. Omnibus test for main effects and interactions of control mouthwash (rinse A) and test mouthwash (rinse X) on implant and non-implant sites.

Sites of Implants responding differently to treatment

All outcomes: measures in table

Transformed Variable: Average outcomes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	2790.339	1	2790.339	9371.807	0.000
Implant	2.916	1	2.916	9.793	0.002
Measure	13.625	1	13.625	45.761	0.000
Group	1.954	1	1.954	6.562	0.011
Implant * measure	7.58	1	7.58	25.459	0.000
Implant * group	0.018	1	0.018	0.061	0.805
Measure * group	8.331	1	8.331	27.981	0.000
Implant * measure * group	16.127	1	16.127	54.166	0.000
Error	60.738	204	0.298		

Implants and Probing depth

Probing depth scores (PDS) were only collected at initial and last observations visits 1 and 4. At intake, control group (Group A) patients had significantly higher levels of probing depth around implanted teeth (mean = 2.93, SEM = 0.094) than in non-implanted teeth (2.69, 0.063), with $t = 2.31$, df = 53, $p = 0.025$. In addition, control group (Group A) implant site probing depth was greater at intake than for test group (Group X) probing depth at implanted sites, 3.40 (0.109) versus 2.45 (0.080) respectively, with $t = 7.07$, df = 52, $p < 0.001$.

Table 5 represents the results in an overall test of significance with PDS as a dependent variable. There were two notable interactions. One was a time by group interaction, representing that probing depths changed significantly differently over time. Another was a three-way interaction time-by-implant-by-group assignment. The time-by-group interaction was seen before in the PDS score and while significant, the actual difference was less than 1%. The three-way interaction is a false signal in that implant scores probe scores increased slightly in the test group (Group X) and decreased slightly in the control group (Group A) – and neither change was significant.

Probing depth scores for implant and non-implant sites did not change significantly in both groups did not change significantly in both implant and non-implant sites as observed over time for both groups. Figure 8 illustrates the lack of change in implant and non-implant depth scores in either group.

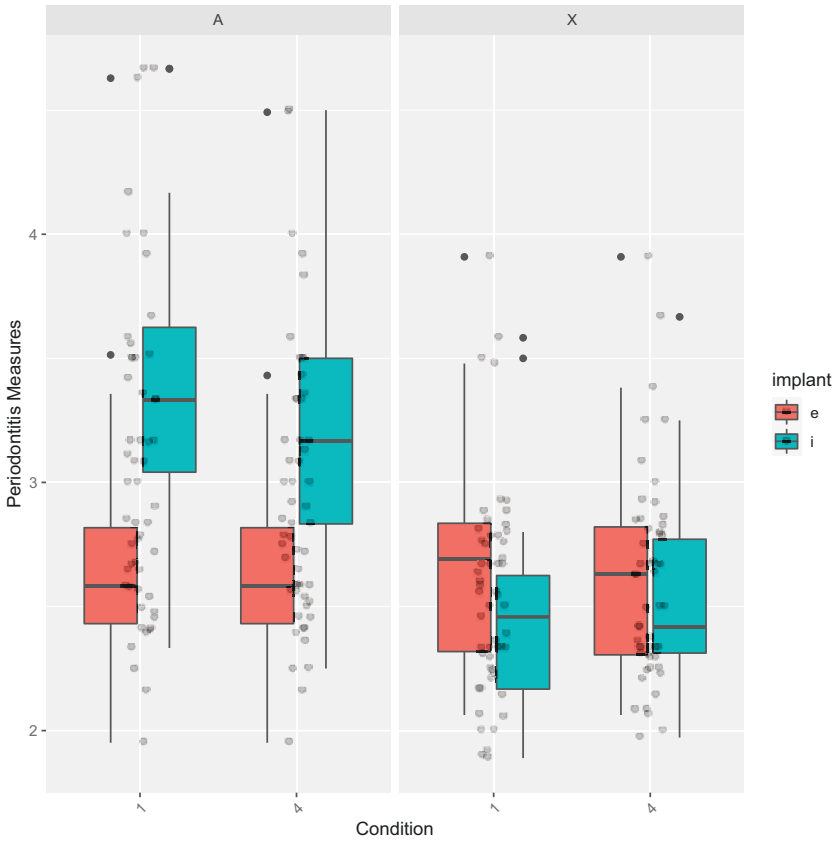
Table 5. Summary results of general linear model of probing depth measures for implant sites over time.

Tests of Within-Subjects Contrasts

Measure: GGI

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	15.113	1	15.113	563.159	0.000
Time * implant	1.507	1	1.507	56.172	0.000
Time * group	0.941	1	0.941	35.047	0.000
Time * implant * group	0.001	1	0.001	0.041	0.840
Error(Time)	2.737	102	0.027		

Figure 8. No differential effects of mouthwashes on probing depth between implant and non-implant sites. Both groups showed only slight decreases in probing depth. Note that in control condition (rinse A), probing depth was higher for the implant group at baseline.



Implants and Gingival Index

Implant versus normal oral sites also showed a significant main effect ($F = 79.0$, $df = 1$, $p < 0.001$) indicating more and faster gingival index improvement in test group (Group X) for both implant and non-implant sites. Test group (Group X) implants decreased in gingival index scores by 83% while implants in control group (Group A) decreased by 57%. The non-implant sites for control group (Group A) decreased by 23% and for test group (Group X) by 50%. The rate of change was faster for test group (Group X) implants than for control group (Group A) and faster that test group (Group X) non-implant sites. Test Treatment (rinse X) members' implants decrease in gingival index measured 26% versus 19% for those in control group (Group A).

Table 6 indicates a strong and statistically significant difference between implant and non-implant sites between and within groups – over time. Figure 9 illustrates the higher gingivitis index in test group (Group X) and greater and faster decrease of gingivitis in test group (Group X).

Table 6. Summary results of general linear model of gingival index measures.

Tests of Between-Subjects Effects

Gingivitis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1207.001	1	1207.001	11073.258	0.000
implant	9.949	1	9.949	91.274	0.000
group	9.177	1	9.177	84.187	0.000
implant * group	8.613	1	8.613	79.021	0.000
Error	11.118	102	0.109		

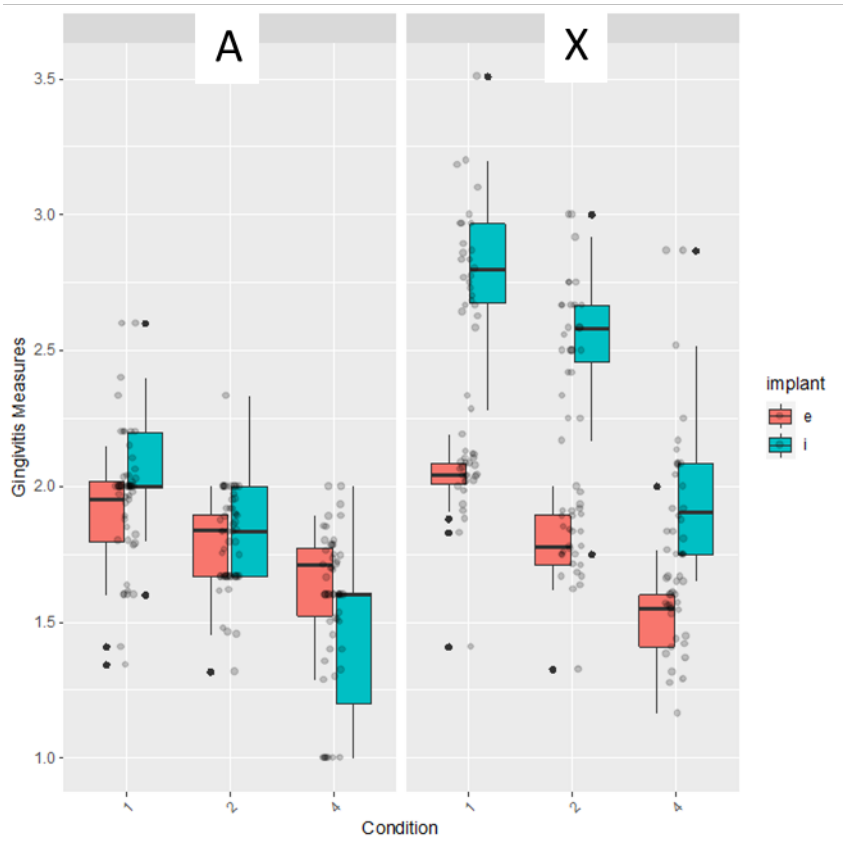


Figure 9. Significant differential effects of mouthwashes on gingival index at implant sites. Both groups showed significant decreases in plaque, but the test group (Group X) reduced gingival index scores more and at a faster rate. Note the difference in gingival index scores at the beginning of the trial.

Implants and Plaque

Plaque scores (PS) for both implant and non-implant plaque scores were higher in the test group (Group X) (2.99 (0.071)) than in the control group (Group A) (2.35 (0.100)) at the first visit, $t = 5.45$, $df = 55$, $p < 0.001$. As with gingival index, PS decreased more for test group (Group X) patients (-0.799 (0.109)) than for control group (Group A) (-0.156 (0.064)) in both implant and non-implant sites, $t = 5.75$, $df = 55$, $p < 0.001$.

Table 7. Summary table of differential effects of control Treatment (rinse A) and test Treatment (rinse X) across implant status and group assignment. The group by implant interaction is significant, indicating the mouthwashes acted differently on implant versus non-implant sites.

Tests of Between-Subjects Effects

Plaque

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1596.963	1	1596.963	3282.732	0.000
implant	0.547	1	0.547	1.124	0.292

group	1.108	1	1.108	2.278	0.134
implant * group	7.532	1	7.532	15.483	0.000
Error	49.62	102	0.486		

Both implant and non-implant sites in the test group (Group X) improved significantly more than control group (Group A), but the implant sites improved significantly more for test group (Group X) (Control group (Group A) slope = -0.151, Test Group (Group X) slope = -0.463, one-analysis of variance $p < 0.001$), a 207% reduction in plaque scores over control group (Group A). Thus, test group (Group X) plaque measures on implant sites improved by 57% more than on non-implant sites. See Figure 10.

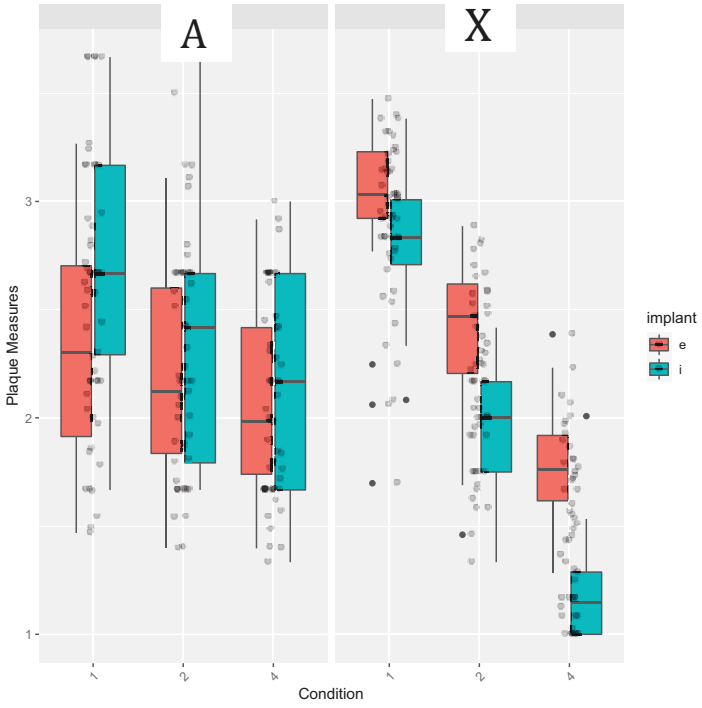


Figure 10. Significant differential effects of mouthwashes on plaque at implant sites. Both groups showed significant decreases in plaque, but test group (Group X) showed significantly greater decrease when testing the difference in slopes.

Conclusions

There is a clear advantage to using ClōSYS® Ultra Sensitive oral rinse treatment (test rinse X) in terms of plaque and gingival index. In addition, ClōSYS® Ultra Sensitive oral rinse (test rinse X) appeared to have a preferential benefit for implant sites, over both control treatment (placebo rinse A) and over non-implant sites. The ClōSYS® Ultra Sensitive rinse (test rinse X) significantly reduced the plaque index and gingival index as compared to initial measures and those in control group (placebo rinse A) ($p < 0.001$). The observation of higher initial gingivitis scores for the ClōSYS® Ultra Sensitive oral rinse (test rinse X) did not affect the superiority of this test group treatment. In controlling for the inequality at baseline, and analysis of covariance showed the reduction of the plaque score remained significantly better in the ClōSYS® Ultra Sensitive oral rinse group (test rinse X) than for the control group (placebo rinse A) ($p < 0.001$). Also, using pre-post difference scores and slopes of the scores over time showed the treatment effect to be robust to the mismatch of initial levels of gingivitis and plaque ($P < 0.001$ for both).

Both groups improved from the use of mouthwashes, but the ClōSYS® Ultra Sensitive oral rinse (test rinse X) improved more and at a faster rate ($p < 0.001$) compared to the control group (placebo rinse A). There was no apparent effect on periodontitis between the two intervals in which it was measured.

Implant sites were significantly more ($p < 0.001$) and faster ($p < 0.001$) improved in the ClōSYS® Ultra Sensitive oral rinse group (test rinse X) than the other non-implanted teeth and in the control group (placebo rinse A).

Both ClōSYS® Ultra Sensitive oral rinse (test rinse X) and placebo rinse (rinse A) tested are safe to oral tissues and did not show any adverse effects.

Both ClōSYS® Ultra Sensitive oral rinse (test rinse X) and placebo rinse (rinse A) tested are safe to oral tissues and do not have any adverse effects.

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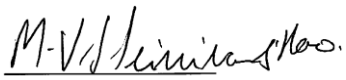

Principal Investigator

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2. Loe H, Silness J. Periodontal disease in pregnancy. *Acta Odont Scand.* 1963;21:533-551.