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Efficacy of ClōSYS® Sensitive Fluoride Toothpaste and Sensitive Rinse Regimen on Plaque, Gingival Inflammation and Bleeding Upon Probing

Final Study Protocol

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Background

Periodontal diseases are mainly the result of infections and inflammation of the gingiva and bone that surround and support the teeth.[1] Bacterial accumulation in a biofilm (dental plaque) that coats the hard and soft tissues of the mouth is the primary causative agent for periodontal disease.[2] Host response, environmental factors (e.g., smoking) and parafunctional habits (e.g. tooth grinding) can all influence the extent and severity of disease and its rate of progression.[2]

Gingivitis is the earliest stage of disease.[1] Patients with gingivitis exhibit classic signs of inflammation, including redness and swelling of the gum tissue, and bleeding upon periodontal probing and/or when performing daily oral hygiene. [2] Regular preventive dental care and proper daily oral hygiene techniques that effectively remove dental plaque can reverse gingivitis. However, if left untreated, gingivitis may progress to periodontitis. [2] Periodontal disease occurs more commonly in adults. Approximately 47% of adults ages 30 years and older have some form of periodontal disease, and prevalence increases with age.[3] Estimates are that 70% of older adults, ages 65 years and older have periodontal disease. [3]

Chemotherapeutic mouthrinses and toothpastes have been shown to be efficacious in helping to remove the biofilm in conjunction with manual plaque removal with toothbrushing, flossing and oral irrigation. [4,5] This study investigated the efficacy of an over the counter (OTC) toothpaste and mouthrinse, both produced according to the FDA's CFR21 monograph for products designed to reduce supragingival plaque and gingivitis, for reducing supragingival plaque, gingivitis, and bleeding on probing. The products used in the study were ClōSYS® Sensitive Fluoride Toothpaste, which contains 0.1% stabilized chlorine dioxide (sodium chlorite in an aqueous solution) and 0.13% w/v fluoride ion, and ClōSYS® Sensitive Rinse which contains 0.1% stabilized chlorine dioxide. Early research supports the efficacy of stabilized chlorine dioxide for reducing oral biofilm.[6] A twice daily oral hygiene regimen using both products was tested in adult subjects diagnosed with gingivitis and early periodontitis.

Objective

The purpose of the study was to evaluate the efficacy of twice daily use of CloSYS Sensitive fluoride toothpaste and Sensitive oral rinse on gingival bleeding upon probing and supragingival plaque in patients with gingivitis or periodontitis (Stage I or II).

Study Design

This was a 90-day, self-controlled, longitudinal, non-blinded clinical trial which measured changes in clinical parameters in participants with gingivitis and Stage I or II periodontitis who applied CloSYS Sensitive fluoride toothpaste twice daily with a toothbrush and CloSYS Sensitive oral rinse twice daily after brushing. Gingivitis was defined as bleeding upon probing with no loss of attachment, and periodontitis was defined when loss of periodontal tissue support through inflammation is the primary feature.

The primary response variables were Subjects' scores on the Plaque Index (PI), Gingival Index (GI) and the number of sites with bleeding upon probing (BOP). Probing depths and clinical attachment level were assessed at baseline and 3 months. Adverse events were assessed and documented at each visit. In addition, Subjects were instructed to keep a usage diary

documenting their daily use of the toothpaste and oral rinse and any comments or observations regarding usage or adverse events.

Number of Subjects
The study was conducted with 60 medically healthy participants between ages 18 to 80 years with gingivitis or periodontitis (Stage I or II) who were receiving routine preventive care and/or periodontal maintenance at the Arizona School of Dentistry & Oral Health, A.T. Still University in Mesa, Arizona.

Inclusion Criteria
The following inclusion criteria were used to determine eligibility for study participation:

- Subjects were between the ages of 18 to 80 years
- Subjects had a minimum of 20 permanent teeth, excluding third molars
- Subjects had been diagnosed with gingivitis or periodontitis (Stages I and II are based on the level of CAL and Bone Loss (BL). The diagnosis was Stage I if: (a) BL is less than 15% and (b) CAL is between 1-2mm. The diagnosis was Stage II if: (a) BL is between 15% and 33% and (b) CAL is between 3-4mm)
- Subjects exhibited bleeding upon probing in >20% of sites
- Subject were seen every 3 months for routine dental prophylaxis/periodontal maintenance
- Subjects did not have a significant medical history or metabolic diseases (diabetes with A1C $\geq$ 7.0, pregnancy)
- Subjects were currently non-smokers or had discontinued smoking at least 6 months prior to enrollment
- Subjects agreed to refrain from use of the following products: Peridex®, PerioGuard®, Listerine®, Cepacol®, Crest Pro-Health® rinse, Colgate Total® rinse, Colgate Total® toothpaste, Crest Pro Health® toothpaste, or any generic equivalent while participating in this study. Alcohol is not the active ingredient in any oral rinse product and does not produce a significant antibacterial effect
- Subjects were able to read and provide written informed consent
- Subjects were able to follow verbal and/or written instructions, perform oral hygiene procedures and return to the test facility for specified study examinations
- Subjects agreed not to receive a dental "cleaning" (prophylaxis) while in the study
- Subjects had to permanently reside in Arizona

Exclusion Criteria
The following criteria were used to exclude study participation:

- Subjects had significant medical history or poorly controlled/uncontrolled diabetes (as defined above)
- Subjects were pregnant
- Subjects had a medical condition that required antibiotic prophylaxis prior to dental treatment
- Subjects had taken antibiotics within 1 month of study enrollment

- Subjects who took multiple medications and/or herbal and dietary supplements known to alter bleeding and exhibits/reports spontaneous gingival bleeding
- Subjects took medications that may interfere with study results (eg. antibiotics, steroids, immunosuppressants, high dose aspirin (325+ mg/day), chemotherapy and/or radiation therapy for cancer).
- Subjects were current smokers
- Subjects had a history of non-surgical or surgical periodontal therapy within 6 months of study participation
- Subjects had Stage III or IV periodontitis, Grade B or C
- Subjects were currently taking or in the past 28 days had taken another investigational drug or participated in other investigational studies that may impact study outcomes
- Subjects had not had a dental cleaning within six months prior to the start of the study

Screening
Potential subjects were identified by the Principal Investigator (PI) following chart review and recruited by the dental hygienist during their routine preventive care (adult prophylaxis) or periodontal maintenance visit. Potential subjects were then evaluated by the PI for additional screening.

The PI reviewed patient charts to determine eligibility for potential participants in the study by eliminating those patients who did not meet the criteria for participation. All patients' periodontal status was assessed from periodontal charting and current radiographs. Only patients without active periodontal disease were considered.

Patients diagnosed with Stage I or II periodontitis who were now stable with 'reduced periodontium' and having regular periodontal maintenance were considered for inclusion. Once the clinical exam was completed and reviewed by the PI was a patient considered for acceptance into the study.

During the screening visit, every potential subject was interviewed and examined by the PI to determine eligibility according to stated inclusion/exclusion criteria. The PI explained the study procedures, with risks and benefits of participation, and the subject was given the opportunity to ask questions.

a. Screening Interview: The Subject was asked about inclusion and exclusion criteria, demographics, smoking or non-smoking, and date of last professional dental cleaning (Appendix A: Screening Form). The following were also reviewed:
    i. Medical History and Medications: A brief medical history for each subject was recorded.
    ii. Dental Health History: The Subject was asked to report the date of their last professional cleaning. Subjects were excluded if they have not had a professional cleaning within six months prior to the start of the study.
b. Oral Examination: The Subject underwent a periodontal examination for PD, CAL and BOP by the hygienist (JB) as part of their professional cleaning visit. Subjects must have had at least 20 permanent teeth to be eligible to participate. The periodontal status of the subject was determined, with eligibility confirmed if diagnosed as having gingivitis or

periodontitis (Stages I and II). Subjects also had to exhibit bleeding upon probing in >20% of sites.

c. Enrollment Determination:
    a. Upon completion of the screening visit, the PI reviewed the screening documents and determined whether or not the potential Subject qualified for study enrollment.
    b. If the Subject qualified, the baseline examination and prophylaxis were performed on the day of the screening examination.

Enrollment

All Subjects who successfully completed the screening were invited to participate. At this time, written informed consent was obtained. The written informed consent document(s) were signed and personally dated by the Subject and completed to a fully executed informed consent document and processed per the institution's standard operating procedures. A copy of the signed informed consent document was provided to each subject upon request.

Subjects who were consented and enrolled in the study were assigned a unique identification number (UIN) which was used to identify the Subject throughout the study and on all applicable study documentation related to that Subject. Subjects' UIN remained constant throughout the study.

Study Procedures

Subjects who were enrolled in the study were scheduled for their Baseline appointment (Visit 1).

A full periodontal examination was performed, including recording probing depths (PD) and clinical attachment loss (CAL) at six sites per tooth for all teeth.

Bleeding on Probing (BOP) was recorded as a dichotomous variable (present/absent) during the periodontal examination. The percentage of sites with bleeding was calculated by dividing the number of sites with bleeding by the total number of sites.

At the Baseline appointment (Visit 1, Day 0), the Subject had a photograph taken of their mouth. The Gingival Index (GI) was measured first using the Modified Gingival Index (MGI).[7,8,9] The severity of gingival inflammation was recorded from the buccal, lingual and interproximal aspects of all evaluable teeth as follows:

- Score 0 = absence of inflammation
- Score 1 = mild inflammation or with slight changes in color and texture but not in all portions of the gingival margin or papilla
- Score 2 = mild inflammation, such as the preceding criteria, in all portions of the gingival margin or papilla
- Score 3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of the gingival margin or papilla
- Score 4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary congestion or ulceration

MGI scores were determined by visual exam of the gingiva on 4 surfaces for each tooth present except for third molars, for a total of 28 teeth. The total number of teeth exhibiting inflammation affecting all portions of the gingival unit (Score ≥ 2) were noted at each visit. The total number of teeth affected was evaluated for each visit in the final analysis.

Next, the Plaque Index (PI) was recorded on all teeth except for third molars, as originally described by Silness and Löe.[10,11] The PI was recorded on 4 surfaces of each tooth and scored as follows:

- Score 0 = No plaque in gingival area
- Score 1 = No plaque visible by the unaided eye, but plaque is made visible on the point of the probe after it has been moved across surface at entrance of gingival crevice.
- Score 2 = Gingival area is covered with a thin to moderately thick layer of plaque; deposit is visible to the naked eye
- Score 3 = Heavy accumulation of soft matter, the thickness of which fills out niche produced by gingival margin and tooth surface: interdental area is stuffed with soft debris

To calculate the Plaque Index (PI), the four scores were summed for each tooth, and then all scores for all of the teeth present in a subject's mouth were added to determine a total score. Then, the total score was then divided by the number of surfaces to yield the percentage plaque score used in the final analysis.

Results of the clinical indices were recorded on the approved data collection form by the PI.

Study products were then dispensed to the subject with both written and verbal instructions for use and the Diary Usage Form. Toothpaste tubes and oral rinse bottles were weighed prior to dispensing, with weights recorded on the data collection form. Dispensed products included two 32-ounce bottles of CloSYS Sensitive oral rinse and two 7-ounce tubes of CloSYS Sensitive fluoride toothpaste and disposable dosing cups. The PI provided both verbal and written oral hygiene instructions as follows:

- Clean between the teeth using dental floss or a proxy brush
- Brush with CloSYS Sensitive fluoride toothpaste for 2 minutes twice daily. Subjects were permitted to brush with either a manual or power toothbrush according to individual preference.
- After brushing, rinse with 15 mL CloSYS Sensitive oral rinse for 30 seconds, then expectorate. Do not eat or drink for 30 minutes after rinsing.

Subjects also received a Diary Usage Form with instructions to complete the form daily. Subjects documented brushing and rinsing behaviors, including the time and date for each brushing/rinsing event. Subjects were also instructed not to use any other oral care products than those given as part of the study during the entire study period. Subjects were instructed to bring back their toothpaste tubes and oral rinse bottles at each subsequent study visit along with the

Diary Usage Form. Self-reported product use and remaining product weight were used to assess compliance with the study regimen.

The subject then received an oral prophylaxis and was scheduled to return in 6 weeks for a follow-up study appointment (Visit 2).

Subjects returned for 2 additional study visits, scheduled every 45 days (Visit 2, Day 45; Visit 3, Day 90).

At Visit 2 (Day 45), subjects received photos and MGI, PI. The presence/absence of adverse events was assessed and documented. The completed Diary Usage Form and remaining products were collected, and products were weighed, with weights recorded on the data collection form. A new Diary form, one new tube of toothpaste, one new bottle of oral rinse and disposable dosing cups were dispensed with instructions to return the form and remaining products at the final study visit (Visit 3).

At Visit 3 (90 days), subjects received photos, MGI, PI, PD, CAL, BOP. Remaining products were collected and weighed, with weights recorded on the data collection form. The Diary Usage Form was also collected. The presence/absence of adverse events was assessed and documented. Subjects completed a brief survey about their product use experience and preferences (ease of use, taste, willing to continue regimen, willing to purchase).

The presence or absence of adverse events (yes/no) was documented at each study visit on the data collection form. Adverse events that occurred were described and recorded by the PI on the Adverse Events form. If indicated, photographs were taken and appropriate reporting was made to the ATSU IRB and Study Sponsor according to the study protocol and university policies.

For Visits 2 and 3, compliance was scored as a dichotomous variable (yes/no). Compliance was scored as "yes" if product weights decreased upon return and if self-reported product use according to the study protocol was completed 90% of the time as documented on the returned Diary Usage Form.

At the end of the final study visit, each subject who completed the study in its entirety was given a $50 Amazon gift card as a reward for successful study completion.

Summary of Study Visits

- Screening exam/Consent
- Visit 1(0 Days) = Baseline
    - ✓ Updated medical history
    - ✓ Photos/Indices (MGI, PI, PD, CAL, BOP)
    - ✓ Dispensed Products
    - ✓ Provided Verbal and Written Oral Hygiene Instructions and Diary
- Prophylaxis provided by dental hygienist
- Visit 2 (45 days)
    - ✓ Updated medical history
    - ✓ Photos/Indices (MGI, PI)

- ✓ Assessed adverse events
- ✓ Collected unused product which was weighed for compliance
- ✓ Collected Diary was reviewed for compliance
- ✓ Dispensed Products and Diary

- Visit 3 (90 days)
  - ✓ Updated medical history
  - ✓ Photos/Indices (MGI, PI, PD, CAL, BOP)
  - ✓ Assessed adverse events
  - ✓ Collected unused product which was weighed and reviewed for compliance
  - ✓ Collected Diary was reviewed for compliance
  - ✓ Subject product use experience and preferences survey completed
  - ✓ Dispensed $50 gift card reward

Removal of Subjects
Subjects were given the right to withdraw from the study at any time and for any reason without prejudice to receiving future dental care at the institution. The PI documented the withdrawal/removal from the study and the reason for withdrawal/removal in the study record. If a subject experienced a complication and wanted to withdraw, if they consented, they were scheduled for an immediate follow-up/closeout examination to determine if an adverse event was present, and if so, the causation of the adverse event was recorded. Every effort was made to follow-up with subjects who withdrew on their own from the study.

Subject data only up to the time when the subjects were withdrawn was kept but was not included in the data analysis of the study.

Off Study
Subjects were considered off study when all planned study visits, early termination, and follow-up visits were completed.

Study Outcomes
This study assessed differences in clinical outcomes at baseline, 6 and 12 weeks. The primary outcomes include changes in gingival and plaque indices (GI and PI, respectively), and bleeding on probing. Because enrolled subjects presented primarily with gingivitis, changes in pocket depth and clinical attachment level were not included in the initial data analysis, as clinically, changes observed were negligible. Secondary outcomes included adverse events, compliance with the oral hygiene regimen and patient product use experience and preferences data.

14. Hypotheses
The hypotheses for the study were as follows:

- Subjects who apply CloSYS Sensitive fluoride toothpaste and Sensitive oral rinse as directed will experience reductions in PI across time.
- Subjects who apply CloSYS Sensitive fluoride Toothpaste and Sensitive oral rinse as directed will experience reductions in GI across time.
- Subjects who apply CloSYS Sensitive fluoride Toothpaste and Sensitive oral rinse as directed will experience reductions in BOP across time.

Statistical Analysis

Data was coded and entered into an Excel spreadsheet which was then imported into IBM SPSS version 28 for analysis. Descriptive statistics were used to report demographics. The Kruskal-Wallis test, with the Dunn post-hoc test, was used to compare MGI and PI at baseline, 6 weeks and 12 weeks. Pairwise comparisons using Wilcoxson signed rank test was used to compare differences in bleeding on probing (BOP) between baseline and 12 weeks. Adverse events data, compliance data and patient product use experience data were reported using descriptive statistics. All data were de-identified and reported in aggregate form only.

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