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EVALUATION OF THE FLUORIDE DOSE RESPONSE OF MFP DENTIFRICE USING IN SITU CARIES MODEL

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Sponsor:	The Procter & Gamble Company Worldwide Clinical Investigations—Oral 8700 Mason-Montgomery Road Mason, OH 45040

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Signatures below indicate approval of the Protocol Amendment 1

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE(s)	Adverse Event(s)
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
B&A	Balance & Assignment
CFR	Code of Federal Regulations
CRF(s)	Case Report Form(s)
ECRF	Electronic Case Report Forms (system)
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IRB/IEC	Institutional Review Board/Independent Ethics Committee
ICH	International Council for Harmonization
ITT	Intent-to-Treat
MFP	Sodium Monofluorophosphate
PP	Per Protocol
SAE	Serious Adverse Events
SMH	Surface Microhardness
SnF_2	Stannous Fluoride
OSHT	Oral Soft/Hard Tissue Exam
OST	Oral Soft Tissue
EFU	Enamel Fluoride Uptake

1. CLINICAL STUDY INFORMATION

1.1 Background

Fluoride toothpaste is the most widely used form of fluoride delivery worldwide. Fluoride dentifrices have shown in numerous clinical trials to be effective anticaries agents and have been the subject of several systematic quantitative evaluations (Marinho et al., 2003; Twetman et al., 2003), which provide the highest standard of evidence for the effectiveness of fluoride dentifrice. Marinho et al. (2003) based their conclusions on a meta-analysis of 70 trials of the effectiveness of fluoride dentifrice for the prevention of dental caries in children compared to placebo. They found evidence that the use of fluoride dentifrices has a caries-inhibiting effect (average reduction in Decay, Missing, and Filled Surfaces (DMFS) of 24%) on the permanent dentition. In addition, they concluded that the effectiveness of fluoride dentifrice may be relatively greater in individuals with a higher caries experience, with increased fluoride concentration, increased frequency of use, and with supervised brushing. There was no evidence that the effect was dependent on background exposure to fluoridated water. Twetman et al. (2003) reached similar overall conclusions from their systematic review.

The current levels of fluoride dentifrice products that are marketed worldwide generally fall in the range between 1000 – 1500 ppm F, although dentifrices with lower fluoride concentrations are marketed in some countries. All fluoride toothpaste sold in the US is in the 1000-1100 ppm F range. Walsh et al. (2010) reported based on a network meta-analysis that the relative anticaries effects of fluoride toothpastes increased with higher fluoride concentration. Based on 74 trials involving the caries scores (DMFS) in the mixed or permanent dentition, the anticaries effect of fluoride toothpaste was 23% for 1000/1055/1100/1250 ppm F and 36% 2400/2500/2800 ppm F; however, toothpastes with 440/500/550 ppm F and below did not show a statistically significant effect compared to placebo.

Clinical caries trials remain the standard for evaluating the safety and efficacy of fluoride dentifrice products. However, due to the high cost involved in conducting clinical caries trials, several surrogate measures of fluoride efficacy have been introduced. These include in situ caries models, rat caries models, in vitro demineralization and remineralization studies, and fluoride uptake studies. These model systems have been extensively reviewed at a Conference held in Rochester, NY in 1994 (Adv Dent Res 9:169-340, 1995). While intra-oral models have been in use for the past forty years, a "Consensus Conference of Intraoral Models" (ADA, Chicago, Sept. 1990) clearly identified the need for validation of intra-oral caries models for their potential use as methods of evaluating the efficacy of fluoride dentifrices and other fluoride-containing dental products. Based on this conference and the Rochester Models conference, there is general agreement among researchers in the field that appropriately validated in situ models represent an acceptable approach for testing the anticaries potential of fluoride products. The use of intraoral appliance models overcome the ethical and practical problems associated with human studies by permitting the evaluation of subtle changes in the mineral status of enamel specimens occurring at a well-controlled test site mounted in the subject's own partial denture. Intraoral models serve as bridges between the clinical situation and the more controlled laboratory environment. Philosophically, these models should, as far as possible, be representative of what occurs in the clinical situation, yet keep variables under control so that meaningful outcome differences can be detected in a reasonable number of subjects (Zero, 1995).

Previous work by our group has shown that a modification of the Koulourides intra-oral model (Koulourides et al., 1974) has sufficient sensitivity and reproducibility to respond in dose-response manner to meet the requirements for model validation (Proskin et al., 1992). The model, which uses partially demineralized enamel as the starting hard tissue substrate, permits the evaluation of the ability of the test dentifrice to enhance net remineralization and measure fluoride bioavailability under clinically relevant conditions. Our current model has been validated based on its response to different dentifrice fluoride concentrations - 0, 250, 500 and 1100 ppm fluoride (Zero et al., 1994; Zero, 1995; Zero et al., 2005) as well as in several more commercially funded in situ studies testing fluoride dentifrice products that also included fluoride dose response controls (Zero et al., 2018).

Our intraoral model system uses the surface microhardness (SMH) test to assess changes in the mineral content of enamel. A very precise instrument (Wilson 2100 Hardness Tester) with a Knoop diamond under a fixed load is used to place indentations into the enamel surface. The length of the indentations is accurately measured using an image analysis system and correspond directly to the depth of

penetration of the diamond into the enamel surface (30:1 ratio). This method can be considered analogous to the dentist's use of a sharp dental probe to detect clinical dental caries; however, the SMH test is hundreds-fold more sensitive and accurate. Using the SMH test, the hardness of sound enamel can be measured and compared with the hardness of enamel after exposure to a caries (demineralization) challenge and after exposure to conditions that favor remineralization. An increase in the indentation length compared to the baseline indentation length indicates softening of the enamel and demineralization. A decrease in the indentation length represents rehardening of the enamel surface and remineralization. The SMH test is one of the few methods that can be used to accurately determine the very early changes occurring at the enamel surface during the caries process (Zero, 1995). For early enamel lesions, a decrease in SMH has been shown to be linearly related to mineral loss (demineralization) (Zero et al., 1990). White (1988) reported that for shallow enamel lesions, an increase in SMH was highly correlated with remineralization as measured by microradiography.

Another advantage of our in-situ model system is that fluoride uptake can be measured on the same enamel specimens that are used to evaluate changes in mineral status (demineralization/remineralization) of enamel. Fluoride uptake testing using in vitro models is accepted by the American Dental Association as one of the profile methods for generic efficacy testing of dentifrices in their Seal Acceptance Program and by the Food and Drug Administration as one of the testing procedures for fluoride dentifrice drug products in the Final Monograph - Anticaries Drug Products for Over-the-Counter Human Use. An important advantage of determining F uptake using an intraoral appliance model is that studies are conducted under clinically relevant conditions, which very closely approximate the in vivo situation. Human subjects apply the test dentifrice by brushing their teeth during the intraoral study. The fluoride is delivered in the presence of physiologically secreted saliva and there are intermittent cycles of demineralization and remineralization during the experimental period as with the natural caries process. Furthermore, when the active fluoride agent in a dentifrice is sodium monofluorophosphate (MFP), plaque and saliva present in the oral environment provide the necessary phosphatases to hydrolyze MFP to release free fluoride ion (Pearce and Dibdin, 1995). This will take place only to a limited extent when using non-biologic in vitro testing methods. Thus, the determination of intraoral fluoride uptake should provide a better estimation of the true fluoride bioavailability of fluoride dentifrice products than determination of fluoride uptake in vitro.

1.2 Investigational Products

- 0 ppm F toothpaste and Oral-B® Indicator soft toothbrush.
- 250 ppm F sodium monofluorophosphate toothpaste and Oral-B® Indicator soft toothbrush.
- 1100 ppm F sodium monofluorophosphate toothpaste and Oral-B® Indicator soft toothbrush.
- 2800 ppm F sodium monofluorophosphate toothpaste and Oral-B® Indicator soft toothbrush.
- 1100 ppm gluconate-stabilized SnF_2 toothpaste and Oral-B® Indicator soft toothbrush.

1.3 Summary of Findings

Previous in-situ caries model dose response studies have evaluated the dose response of different dentifrice fluoride concentrations through the use of this in situ caries model.

1.4 Known Potential Risks and Benefits

This study is for research purposes only. There may be no direct benefit to the subjects from participation in the study. Others may benefit from the knowledge gained. There may be other risks of study participation that are unknown. There is no guarantee that the subjects will receive any medical or dental benefits from participating in this research study.

1.5 Investigational Product Dosage

Each subject will be randomized to each of the treatment groups below:

- 0 ppm F toothpaste
- 250 ppm F sodium monofluorophosphate toothpaste

- 1100 ppm F sodium monofluorophosphate toothpaste
- 2800 ppm F sodium monofluorophosphate toothpaste

Subjects willing to participate in a fifth leg of the study will receive 1100 ppm gluconate-stabilized SnF_2 toothpaste as the investigational product.

Subjects will be instructed to brush twice daily (morning and at bedtime) with their assigned dentifrice for 21 days following the brushing instructions found in the Appendix. Subjects will be instructed to brush the biting surfaces of their back teeth (but not enamel specimens) with a full ribbon of study dentifrice for one timed minute while wearing their partial dentures, followed by rinsing with the 15 mL of measured tap water for 10 seconds and then expectorating. Subjects who are regular floss users will be allowed to continue flossing; use of any non-study related products will be disallowed. Compliance with brushing procedures will be recorded in subject diaries (see Appendix), as well as any *de novo* or changes in medical conditions, medications or treatments.

1.6 Ethical Compliance

Prior to study initiation, the Investigator must obtain institutional review and approval of the Protocol, the consent form, and other necessary study-related documents in compliance with the US Code of Federal Regulations, Title 21, Part 56 or the ICH-GCP Consolidated Guidelines, Chapter 3 and in compliance with Procter & Gamble SOP CTN-CL-504 ("Institutional Review Board/Independent Ethics Committee Review and Approval"). The Investigator will maintain any original authorization letter(s) and will be available for review by the Sponsor. IRB approval letters should include the study title, Sponsor study number, the address of the IRB, date of request, and the signature of the IRB chairperson or designate. Additionally, the letter must acknowledge that both the Protocol and consent form have been approved by the IRB. The study will not begin until the Sponsor has received confirmation of IRB approval. The IRB shall also review the investigation at least once a year during study execution. The Investigator will notify the IRB when the study is terminated and provide confirmation that the study has been closed with the IRB to the Sponsor.

The Investigator will obtain written informed consent for each subject prior to participation in the study, per the US Code of Federal Regulations, Title 21, Parts 50.25 and 50.27 and ICH-GCP, Chapter 4, subpart 4.8 and in compliance with Procter & Gamble SOP CTN-CL-503 ("Informed Consent Form, Ethics Approval and Investigator Use"). Subjects, or their legal guardian, are required to read, sign and date an IRB approved consent form with the Investigator also maintaining a signed and dated copy. The subject or legal guardian will be given a copy of the consent form. All study procedures must be explained in non-technical terms.

Changes to the Protocol following IRB approval affecting the safety of subjects, scope or objectives of the investigation, or the scientific quality of the study will be documented as amendments. Such changes will require the Sponsor, Investigator, and IRB approval prior to implementation, unless immediate action is required to safeguard subject safety. Administrative or minor changes (e.g., typographical errors, changes in Sponsor personnel, etc.) will be documented as revisions but may not need to be submitted as amendments unless required by the IRB. Any change in the Sponsor's monitoring staff, Clinical Trial Manager or Medical Monitor during the conduct of the study, will be reported to the Investigator.

During the course of the trial, the clinical site will allow monitoring by the Sponsor (Clinical Trial Manager or designee) to check compliance with the Protocol, regulations and guidelines, adequacy of the equipment and facilities, and satisfactory data collection.

1.7 Target Population

The target population of this study is generally healthy subjects ranging from 18-85 years with a normal salivary flow rate.

1.8 Literature and Data

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2. INVESTIGATIONAL PLAN

2.1 Overall Study Design and Plan

The purpose of this study is to evaluate the fluoride dose response of different dentifrice fluoride concentrations - 0, 250, 1100 and 2800 ppm fluoride as sodium monofluorophosphate (MFP) using an in-situ caries model.

The purpose of Period 5 is to evaluate the performance and sensitivity of the in-situ caries model to a SnF_2 toothpaste.

2.1.1 Primary Endpoints

The primary endpoint is the enamel fluoride uptake on Day 21 and the primary comparison between pastes with 0 and 2800ppm fluoride concentrations, and all other treatment comparisons and time points being of secondary interest. The secondary endpoints include % SMH recovery and SMH change (see 4.4),

2.1.2 Design Type

This is a randomized, four-treatment, 4-period crossover, double blind clinical trial. The target population is approximately 16 adult volunteers. During the course of the study, all subjects will be asked to voluntarily participate in a fifth period, not included as part of the crossover design. Those subjects willing to participate in Period 5 will sign an amended informed consent form and will all receive the same product during that period. Two to three days before the start of each treatment period the subjects will have their teeth cleaned to remove all accessible plaque and calculus and will be provided with a non-fluoride dentifrice to use until their next visit. At the beginning of each testing period, two gauze-covered 4 mm round partially demineralized human enamel specimens will be placed in the buccal flange area of the subject's mandibular partial denture. In addition, two gauze-covered 4 mm round partially demineralized human enamel specimens will be placed in the buccal surface of two posterior denture teeth of the same side of the partial denture. Once specimens are placed, subjects will wear their partial dentures twenty-four hours a day and use their assigned toothpaste twice daily, as instructed, until their next visit. Subjects will return at 7 and 14 days to have one specimen removed per visit, and again at 21 days to have the remaining two specimens removed. The specimen removal sequence will occur in a predetermined order starting with the anterior denture tooth specimen, posterior denture tooth specimen, and then the two buccal flange specimens. Following the 21-day specimen removal visit, the subjects will undergo a four- to five-day washout period followed by another cleaning and two- to three-day lead in period. This process will be repeated until all subjects have used all four test products. Changes in the mineral content of the enamel specimens will be assessed using the SMH. Enamel fluoride uptake (EFU) will be determined using the microdrill enamel biopsy technique.

Table 1. Study Schedule by Procedure Type and Visit

PROCEDURE	VISIT 1	VISIT 2, 7, 12, 17, 22	VISIT 3, 8, 13, 18, 23	VISIT 4, 9, 14, 19, 24	VISIT 5, 10, 15, 20, 25	VISIT 6, 11, 16, 21, 26
	SCREENING	PROPHY	BEGIN TREATMENT	7 DAY REMOVAL	14 DAY REMOVAL	END[1] TREATMENT
Informed Consent	X					
Medical History Review	X	X	X	X	X	X
Demographics	X					
Oral Soft Tissue Exam	X	X	X			X
Oral Hard Tissue Exam	X	X (v2 only)				X (v21 and v26)
Salivary Flow	X					
Inclusion/Exclusion Criteria	X					
Continuance Criteria		X	X	X	X	X
Randomization			X (v3 only)			
Dental Prophylaxis		X				
Placement of specimens			X			
Issue washout toothpaste		X				
Return washout toothpaste			X			

Issue study products and diary			X			
Return study products and diary						X
General Comments	X	X	X	X	X	X
Adverse Events		X	X	X	X	X
Subject Accountability						X[1] (v21 or 26)

[1] For those subjects continuing on Period 5, end of study activities will occur at Visit 26

2.1.3 Mitigating Bias

Subjects will be randomly assigned to a treatment sequence (see section 3.1.2). Identities of the study investigational products will be disguised with a study label. Similar sized kit boxes will be used to ensure product blinding. All treatment products will be dispensed in a secluded area, away from the study examiner, to maintain the study blind. All subjects will receive the same product for Period 5.

The treatment product(s) each subject will receive will not be disclosed to the Principal Investigator, examiners, study center personnel, subjects, contract monitors, contract vendors, or the Sponsor, except for select site personnel responsible for usage instruction and supervised use. Period 5 product will be blinded to analytical personnel.

2.1.4 Labeling and Shipping

The identity of the toothpaste will be disguised. Subjects will receive a kit box containing 1 tube of toothpaste, a timer, a toothbrush and product use instructions to be used throughout the duration of their study period. Subjects will receive a new kit box at the beginning of each study period.

The kit boxes will be labeled by letter code (except Period 5). Kit box labels will also contain the study number, emergency phone number, distributor name/address, appropriate caution statements, content statement and other information as required by internal regulations and clinical SOPs. The shipping containers will be labeled with the clinical site address and a content statement listing study number and kit box numbers contained within. Supplemental products will be provided to site.

The site will be provided with a code breaker report in a sealed envelope. The sealed code breaker report contains documents that list the kit box number or treatment code while the identity of the treatment products is hidden by an opaque scratch-off seal (except Period 5). If the study blind needs to be broken, an individual subject's investigational product may be ascertained by opening the sealed code breaker report, locating the subject's kit box number or treatment code and scratching off the opaque seal to reveal the treatment identity. The sealed code breaker report will be opened if a clinically serious AE occurs or management of the subject requires knowledge of the identity of the investigational product. The Investigator should immediately inform the Sponsor that the code will be broken and record the date, time and reason for breaking the code in writing. After the study is complete and the study database has been finalized and locked, the site will return the code breaker report to the Sponsor using the self-addressed, stamped envelope provided by the Sponsor.

2.1.5 Duration of Subject Participation

Visit 1 (Screening)

Subjects who have been recruited for the study via a telephone interview will sign in at the study site for this and all subsequent visits. At this time, subjects will be asked to read and sign duplicate copies of an informed consent statement, authorization for the release of health information for research form and demographic form. Demographic information and study entry criteria will be obtained and documented on the appropriate case report form (CRF). Upon review of these documents, an update of their medical

history/medications and the inclusion/exclusion criteria, each subject will be given an oral soft/hard tissue exam (OSHT) prior to their acceptance into the study. Their mandibular partial denture will be examined to determine if specimens can be held in one buccal flange area and the posterior denture teeth of the same side, as previously described. They will then provide a stimulated and unstimulated saliva sample to determine salivary flow rate. If no contraindications to their participation are discovered and the subject meets the study requirements, the subject will be accepted into the study.

General comments, if any, will be recorded on the appropriate CRF.

Visit 2 (Prophy Period 1)

Two to three days prior to the beginning of Treatment Period 1, subjects will return for a professional dental prophylaxis. Subjects will answer have their medical history/medication information updated and answer continuation questions. Subjects will then receive an oral soft tissue exam (OST) and oral hard tissue exam (at the first period only). The subject's mandibular partial denture will be prepared to hold the round enamel specimens by drilling out hollows in the partial dentures at the specimen sites in the buccal flange area and the buccal surface of two denture teeth on the same side of the partial denture. A temporary dental material (DentuSil™) will be placed in the drilled-out areas of the partial denture. Subjects will be given fluoride-free toothpaste to use at home for the next two to three days until they return for their next visit. They will be encouraged to remove their partial denture at night until their next visit.

Visit 3 (Begin Treatment Period 1)

Each subject will receive an OST examination, answer continuation questions and have their medical history/medication information updated. Subjects will be asked to return the washout product they received at Visit 2 and will be given their randomized study product and diary for product use. Subjects will be instructed on the brushing method (see Appendix). The four enamel partially demineralized specimens will be placed in the buccal flange/posterior teeth areas on one side of the subject's mandibular partial denture. All subjects will be instructed to wear their partial denture containing the enamel specimens 24 hours a day except during the cleaning of their natural teeth (twice per day) and for short periods to rinse their mouth out with tap water after meals and snacks.

Visit 4 (7 Day Removal)

Seven (7) days following Visit 3, the subjects will return to the Institute and answer continuance criteria questions and update their medical history/medication information. One enamel specimen will be removed from anterior denture tooth site of their partial denture and the hollow will be filled with a temporary dental material (DentuSil™). The subjects will be told to continue using their assigned product as instructed.

Visit 5 (14 Day Removal)

Fourteen (14) days following Visit 3, the subjects will return to the Institute and answer continuance criteria questions and update their medical history/medication information. One enamel specimen will be removed from posterior denture tooth site of their partial denture and the hollow will be filled with a temporary dental material (DentuSil™). The subjects will be told to continue using their assigned product as instructed.

Visit 6 (End Treatment Period 1)

Twenty-one (21) days following Visit 3, the subjects will return to the Institute and receive an OST examination, answer continuance criteria questions and update their medical history/medication information. Subjects will be asked to return the study product and diary they received at Visit 3. The remaining two enamel specimens in the buccal flange site will be removed from their partial denture and the hollows will be filled with a temporary dental material (DentuSil)

Period 2, 3, 4 & 5

The procedures outlined above (Visit 2-6) will be repeated until each subject has used each of the four test products, followed by a 5th test product in Period 5. At Visit 21 (and also at Visit 26 for those subjects

participating in Period 5), subjects will receive an OSHT examination, have their mandibular partial denture cleaned through sonication, if applicable, have their partial denture repaired (if not going on to another in situ study) and their participation in the study will end.

Subject Accountability

If, for any reason, a subject does not complete the study, an explanation will be entered on the Subject Accountability CRF. All data gathered on the subject prior to discontinuation will be made available to the Sponsor.

General Comments and Adverse Events

General comments can be recorded at any time during the study. Adverse Events (AEs) will be documented on the AE CRF. Any self-reported AE that remains unresolved by the end of the study should be followed up until resolution by the investigator/designee, and the resolution should be documented only as source documentation. If a subject is unreachable to determine whether the AE has been resolved, the attempts to contact the subject should be documented as source documentation. Examiner observed AEs that are unresolved at the end of the study are followed to resolution at the discretion of the Medical Monitor.

2.1.5.1 Lifestyle Requirements

1. For 2-3 days following each cleaning visit subjects must discontinue all regular oral hygiene practices (products and procedures) and use only the study fluoride-free toothpaste and toothbrush provided, twice daily, with the exception of interdental cleaners, e.g., dental floss, if this is their normal practice.
2. For the 21 days of each treatment period, subjects may use only the study product assigned to them and the provided toothbrush twice daily, after breakfast and just before going to bed. Subjects may floss if this is their normal practice.
3. Subjects must wear their lower partial denture 24 hours a day, except when cleaning it, during each 21-day treatment period. Subjects will be encouraged to remove their mandibular partial denture at night during the washout and lead in periods.
4. Subjects may use a non-zinc fixative like Poligrip® on their upper denture but no adhesive is permitted in the lower partial denture.
5. Subjects must refrain from eating canned sardines during the course of the study and may not eat hard candy or nuts when the specimens are in place.

2.1.6 Discontinuation Criteria

Discontinued subjects are those who do not complete final evaluations and/or procedures outlined in the protocol because of one of the following:

a) an AE;
b) significant protocol deviation that, in the opinion of the Investigator, may compromise the study results;
c) voluntary withdrawal; or
d) withdrawal at the Investigator's discretion.

The Investigator/Sponsor may recommend dropping a subject from the study at any time. Recommendations to drop a subject may include but are not limited to: misuse of study product, not following study procedures, or a serious protocol violation. Any subject who is discontinued from the study that has a continuing AE will have follow-up communications about the AE until the AE is resolved to the satisfaction of the Investigator/Medical Monitor.

2.1.7 Product Accountability

Study products will be stored in a secure area, under environmental condition as required by label instructions or as described in this protocol and dispensed only under the authorization of the Investigator. The storage condition shall be properly documented. The study site will record and log both the receipt and dispensation of all test products (used and unused) by using forms provided by the

Sponsor or suitable forms provided by the site. Study products will be returned to the Sponsor following the trial, or alternatively, they will be destroyed at the clinical site provided the site has an existing SOP for the destruction of clinical materials and prior written approval from the Sponsor.

2.1.8 Treatment Codes

The site will be provided with a code breaker report in a sealed envelope (except for Period 5). The sealed code breaker report contains documents that list the kit box number or treatment code while the identity of the treatment products is hidden by an opaque scratch-off seal. If the study blind needs to be broken, an individual subject's investigational product may be ascertained by opening the sealed code breaker report, locating the subject's kit box number or treatment code and scratching off the opaque seal to reveal the treatment identity. The sealed code breaker report will be opened if a clinically Serious Adverse Event (SAE) occurs or management of the subject requires knowledge of the identity of the investigational product. The Investigator should immediately inform the Sponsor that the code will be broken and record the date, time and reason for breaking the code in writing. After the study is complete and the study database has been finalized and locked, treatment codes will be unblinded.

2.1.9 Source Data

The Investigator has the responsibility for ensuring that all source documents (i.e., study and/or medical records) and CRFs are completed and maintained according to the study protocol and is available at the site. Any CRF used as a source document must be identified as such in the Investigator Notebook.

2.2 SELECTION AND WITHDRAWAL OF STUDY POPULATION

2.2.1 Inclusion Criteria

In order to be included in the study, each subject must:

i. Be between 18 and 85 years of age;
ii. Provide written informed consent prior to participation and be given a signed copy of the informed consent form;
iii. Be in general good health as determined by the Investigator based on a review of the health history/update for participation in the trial;
iv. Be wearing a removable mandibular partial denture with sufficient room in one posterior buccal flange area to accommodate two 4 mm round enamel specimens and room on the same side to accommodate two 4 mm round specimens in the buccal surface of two posterior denture teeth;
v. Be willing and capable of wearing their removable partial denture 24 hours a day for four (4), three-week treatment periods;
vi. Be willing to allow study personnel to drill specimen sites (as described in #iv) in their mandibular partial denture;
vii. Be in good medical and dental health with no active caries or periodontal disease (NOTE: subjects presenting at screening with caries may continue in the study if their carious lesions are restored prior to beginning treatment 1);
viii. Have a salivary flow rate in the range of normal values (unstimulated whole saliva flow rate ≥ 0.2 mL/min; gum base stimulated whole saliva flow rate ≥ 0.8 mL/min).

2.2.2 Exclusion Criteria

Subjects may be excluded from study participation due to:

i. Currently being pregnant, intending to become pregnant during the study period, or breast feeding;
ii. Currently having any medical condition that could be expected to interfere with the subject's safety during the study period;

iii. Currently taking antibiotics or having taken antibiotics in the two weeks prior to beginning treatment 1;
iv. Having participated in another clinical study or receipt of an investigational drug within 30 days of beginning treatment 1; or
v. Taking fluoride supplements, required to use a fluoride mouthrinse, or have received a professional fluoride treatment in the two weeks preceding specimen placement;
vi. Currently taking or have ever taken bisphosphonate drugs (e.g., Fosamax, Actonel and Boniva) for the treatment of osteoporosis.

2.2.3 Continuance Criteria

Subjects may be excluded from study participation and/or the analysis due to:

i. Use of any non-study oral hygiene products since the previous study visit;
ii. Having any diseases or condition that might interfere with the safe participation in the study;
iii. Having an inability to undergo study procedures;
iv. Significant protocol deviation that, in the opinion of the Investigator, may compromise the study results;
v. Use of antibiotics since the previous study visit;
vi. Being unwilling or incapable of wearing their removable partial denture 24 hours a day for a fifth, three-week treatment period (asked at Period 5, Visit 22 only).

2.2.4 Subject Withdrawal Criteria

Subject participation is strictly voluntary, and a subject may withdraw from the research study at any time. Subjects may withdraw or take away permission to use and disclose health information at any time by sending written notice to the study doctor. If a subject withdraws permission, the subject will not be able to continue being in the research study. When permission has been withdrawn, no new health information will be gathered after that date. Information that has already been gathered may still be used and given to Sponsor.

The Investigator/Sponsor may recommend dropping a subject from the study at any time. Recommendations to drop a subject may include but are not limited to: misuse of study product, not following study procedures, or a serious protocol violation.

Subjects that withdraw or drop from the research study will not be replaced.

If, for any reason, a subject does not complete the study, an explanation will be entered on the Subject Accountability CRF. All data gathered on the subject prior to discontinuation will be made available to the Sponsor.

3. TREATMENTS

3.1.1 Product Usage

Washout Product:
Subjects will be instructed to brush twice daily (morning and at bedtime) with their washout toothpaste and toothbrush following the brushing instructions found in the Appendix.

Treatment Product:
Subjects will be instructed to brush twice daily (morning and at bedtime) with their assigned dentifrice for 21 days following the brushing instructions found in the Appendix. Subjects will be instructed to brush the biting surfaces of their back teeth (but not enamel specimens) with a full ribbon of study dentifrice for one timed minute while wearing their partial dentures, followed by rinsing with the 15 mL of measured tap water for 10 seconds and then expectorating. Subjects who are regular floss users will be allowed

to continue flossing; use of any non-study related products will be disallowed. Compliance with brushing procedures will be recorded in subject diaries (see Appendix), as well as any *de novo* or changes in medical conditions, medications or treatments.

3.1.2 Method of Assigning Subjects to Treatment Groups

Study Design	n	Number of Treatment Periods	Treatment Sequences
Crossover	16	4	ABCD, BDAC. CADB, DCBA

Treatment Sequence Schedule
Eligible subjects will be randomly assigned to one of the 4 pre-specified treatment sequences. Subjects will be assigned to a treatment sequence in the order they come to the site for their first treatment period using a computer-generated treatment sequence schedule. The site will keep the treatment sequence schedule while the study is on-going. Subjects will complete the treatment sequence in the order assigned to them. Should a subject miss a treatment period, that treatment in the sequence will be skipped.

Period 5: The crossover design does not apply to Period 5. All subjects will receive the same product in Period 5.

3.1.3 Prior and Concomitant Therapy
Medical history and concomitant medication will be recorded as source documentation.

3.1.4 Treatment Compliance
Product use compliance will be assessed by determining the average weight of each type of test product prior to dispensing (five tubes per product will be weighed and average weight determined). At the end of each treatment period, subjects will be required to return all product containers (including empty ones) to the study site. The tubes will be individually weighed upon return and that number subtracted from the average weight will be recorded on the treatment dispensing log.

The amount of test product used for each study treatment will be compared to daily product usage recorded on the subject's diary. Significant discrepancies will be discussed with the study subject, as needed. Subjects who miss 15% or more treatments within the three-week treatment period will be considered non-compliant. The reason for non- compliance will be noted in the subjects' study records. All subjects will be instructed to use only the assigned products in place of normal oral hygiene for the duration of the study.

3.2 Assessment of Efficacy

3.2.1 Sample Collection

Salivary Flow Assessment
Salivary flow will be assessed during the screening visit. For the unstimulated collection, subjects will sit quietly for five minutes before beginning the test. During the five-minute test time, they will be told to allow their saliva to pool, emptying into a collection cup whenever they feel the need to swallow.

For the stimulated collections, subjects will chew unflavored gum base for one minute and then swallow any pooled saliva. They will then chew the gum base for two minutes, during which time they will empty any pooled saliva into a collection cup.

The samples will be weighed and the salivary flow rates determined. The unstimulated saliva flow rate must be ≥ 0.2 mL/min and the stimulated saliva flow rate must be ≥ 0.8 mL/min for study qualification.

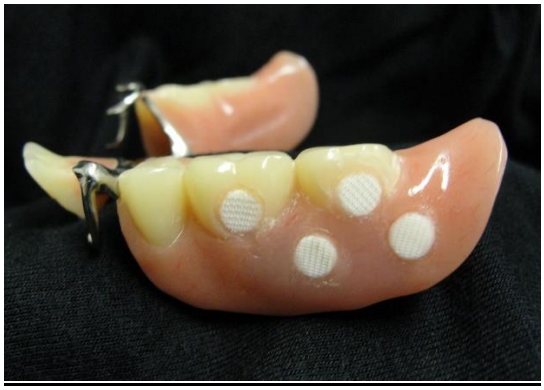
Diary for Home Use
At the start of each treatment period, subjects will be provided with a diary (see Appendix) to record the date, and the time of the morning (a.m.) and evening (p.m.) of each brushing and any deviation from the brushing regimen. In addition, subjects will also record any new or changes in pre-existing medical conditions, medications or non-drug treatments or any change in signs or symptoms that may occur.

Subjects will be required to bring the completed diary to the end of treatment visit. Study staff will review the Diary with the subject to confirm treatment compliance and clarify listed medical conditions, medications and non-drug treatments.

Intra-oral Appliance
The in-situ model involves the placement of gauze-covered enamel specimens in the subject's mandibular partial denture (see Figure 1). The subject's denture will be prepared for the study by creating two hollows in the buccal flange area on one side of the partial denture and in two denture teeth on the same side large enough to accommodate the enamel specimens. Four gauze-covered 4 mm round enamel specimens will be mounted on the partial denture as described previously. The enamel specimens will be mounted in place with DentuSil™ - Silicone Soft Reline Material (The Harry J.Bosworth® Company, Skokie, IL) or an equivalent material. The DentuSil™ material will be placed in the drilled sites and the enamel specimens carefully inserted so that they be mounted flush with the surface of the buccal flange/denture teeth when fully seated. Great care will be taken to avoid contaminating the enamel surface of the specimens with the luting material. Upon completion of the study the subject's partial denture will be repaired. The location where the enamel specimens will be placed on the subject's partial denture are not functional parts of the denture, and the experimental procedures will not cause any permanent damage to the denture.

Prior to placement in the subjects' partial dentures, all enamel specimens will be sterilized by exposure to ethylene oxide.

FIGURE 1
Lower Partial Denture Appliance with specimens placed in the buccal flange and buccal surface of posterior denture teeth



3.3 Assessment of Safety

3.3.1 Safety Variables

Oral Examination
The study dentist will complete an oral soft and hard tissue (OSHT) examination at screening, oral hard tissue (OHT) at Visit 2 and 21(and Visit 26 for those subjects participating in Period 5) and an oral soft tissue (OST) examination at each study visit with the exception of the 7-day and 14-day removal visits.

The exams will be conducted via a visual examination of the oral cavity and perioral area utilizing a standard dental light, dental mirror, gauze, and periodontal probe and tongue blade, as needed. The soft tissue structures examined will involve the labial mucosa including lips, buccal mucosa, mucogingival folds, gingival mucosa, hard and soft palate, tonsillar and pharyngeal areas, Edentulous ridge/Retromolar area, tongue, sublingual area/floor of mouth, submandibular area, major salivary glands, head and neck and TMJ. Observations will be listed as "Normal" and "Abnormal" and abnormalities will be described.

The hard tissue structures examined will include assessing for enamel irregularities, tooth fracture, pathologic tooth wear, cavitated lesions, active non-cavitated lesions, residual roots, faulty restorations and implants. Observations will be listed as "Absent" or "Present" and conditions noted as present will be described.

All abnormal findings are recorded and categorized by their location with hard tissue findings categorized as "other-oral." An AE is recorded if a new abnormal finding is noted after treatment application or any abnormal finding noted prior to treatment application increases in severity after treatment is applied.

3.3.2 Adverse Event Management

An adverse event (AE) is any untoward medical occurrence in a subject administered a product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

A serious adverse event (SAE) is defined as an event which suggests a definite hazard or handicap to the subjects. SAEs are any events resulting in death, life threatening situation, disability or permanent damage, hospitalization or prolongation of existing hospitalization, or congenital anomaly/birth defects; events requiring intervention to prevent permanent impairment/damage; or other serious (important) medical events.

All other AEs that do not fit within these criteria will be considered "non-serious." Unexpected adverse event/reaction is any occurrence the nature or severity of which is not consistent with the AE provided in the current summary of product characteristics. All other AEs are considered "expected."

When an AE is suspected, all relevant evaluations will be carried out and appropriate treatment will be recommended. Additional follow-up will be performed as necessary and recorded as source documentation, with the results provided to the Sponsor. Subjects that experience any clinically significant AE will remain under medical supervision until the Principal Investigator/Medical Monitor recommends appropriate follow-up treatment or deems the AE to be resolved, stabilized, or no longer serious enough to warrant follow-up.

When an Investigator is notified of a serious AE, the Investigator must promptly (within 24 hours) notify the Sponsor (Clinical Trial Manager or the Medical Monitor) of the serious event, regardless of causality. Within 5 working days, a written and/or electronic report describing the circumstances of the event must be submitted to the Sponsor. The Investigator will be responsible for SAE reporting to the IRB.

3.3.3 Adverse Event Reporting

AE Recording

When an AE occurs after written informed consent has been obtained but before the first dose of study drug, the AE will be considered a nontreatment-emergent AE. Only serious nontreatment-emergent AEs related to study procedures will be recorded on the CRF. All nontreatment-emergent AEs that are non-serious or unrelated to study procedures should be documented by updating the medical history and general comments as applicable.

An AE that occurs from the time the subject receives the first dose of study drug until dismissed from the study will be considered a treatment-emergent AE. All treatment-emergent AEs will be collected. The severity, action taken, causality, outcome, and category of all recorded AEs will be recorded.

Severity
Severity refers to the extent to which an AE affects daily activities. Severity will be categorized according to the following criteria:

- Mild: Normal activities unaltered;
- Moderate: Normal activities altered;
- Severe: Unable to undertake normal activities.

The term "severity" is not the same as "serious." Seriousness, not severity, serves as the guide for defining regulatory reporting obligations.

Action Taken
If any action was taken due to the AE, it should be recorded.

- None: No action was taken by the subject.
- Discontinued: Investigator recommended, or subject stopped using the study products.
- Reduced/Interrupted: Investigator recommended or subject reduced or interrupted the instructed product usage.

Causality
Causality refers to the relationship of the AE to study drug. Assessment of causality is the responsibility of the Principal Investigator at each site. If this responsibility is delegated to a sub-investigator, this should be appropriately documented in the delegation sheet. Causality will be categorized according to the following criteria:

- Doubtful: There is no medical evidence to suggest that the AE may be related to study drug usage, or there is another more probable medical explanation.
- Possible: There is medical evidence to suggest that the AE may be related to study drug usage. However, other medical explanations cannot be excluded as a possible cause.
- Probable: There is strong medical evidence to suggest that the AE is related to study drug usage.
- Not Related: There is no evidence to suggest the adverse event is related to study drug usage.

Category
Category refers to the region or area where the AE occurred. Categories are listed 0-9.

- 0 = Non-oral related
- 1 = Perioral area/lips
- 2 = Labial mucosa/buccal mucosa
- 3 = Mucolabial fold/mucobuccal fold
- 4 = Gingiva/free and attached
- 5 = Palate/hard and soft
- 6 = Oropharynx/uvula
- 7 = Tongue
- 8 = Sublingual
- 9 = Other oral

3.3.4 Adverse Event Follow-up

Any self-reported AE that remains unresolved by the end of the study should be followed up until resolution by the investigator/designee, and the resolution should be documented only as source documentation. If a subject is unreachable to determine whether the AE has been resolved, the attempts to contact the subject should be documented as source documentation. Examiner observed AEs that are unresolved at the end of the study are followed to resolution at the discretion of the Medical Monitor.

4. LABORATORY METHODS

4.1 Specimen Preparation

Extracted human teeth will be used as the hard tissue test substrate for the preparation of ~~the 4 x 4 mm and~~ the 4 mm round specimens. The teeth will be collected and transported to OHRI in a saturated thymol solution. Upon receipt, the teeth will be sorted and cleaned. The teeth will then be stored in saturated thymol solution during sample preparation procedures.

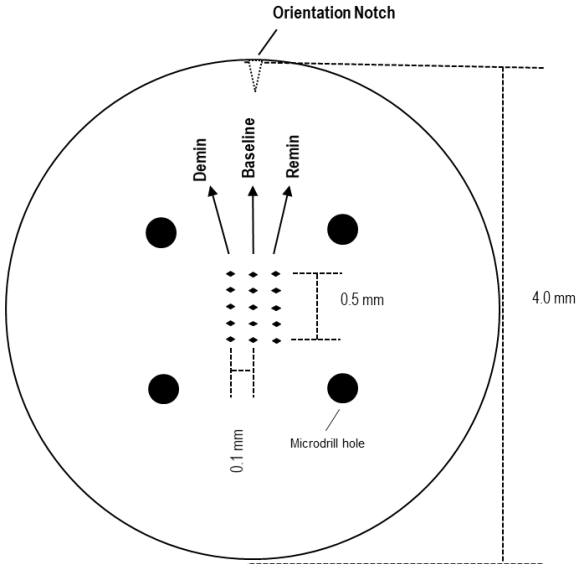
Teeth will be selected based on the following criteria:

- Free of caries and major restorations;
- No discoloration and no markings, such as cracks, when viewed under a microscope at 20× magnification;
- Sufficient tooth surface to provide a large size specimen to meet study requirements.

A core of enamel 4 mm in diameter will be prepared from each human tooth by cutting perpendicularly to the buccal surface with a hollow-core diamond drill bit. Each block will be ground and polished to create flat surfaces [Zero et al., 1990].

The round specimens will be ground and polished to create planar parallel dentin and enamel surfaces. All grinding/polishing will be done on a polishing surface moistened with deionized water (dw). The dentin side will be ground flat using 500 grit silicon carbide paper, followed by grinding and polishing of the enamel side. A small orientation cut will be placed on each block (see Figure 2). The enamel surface of each specimen will be ground using 1200 grit silicon carbide paper followed by 2400 and then 4000 grit silicon carbide paper. After the grinding procedures are completed, the enamel specimens will be placed under running dw for three minutes, sonicated in deionized water (dw) for three minutes and then placed under running dw for three minutes. The polishing step will involve the use of a 1 micrometer (µm) diamond suspension on a polishing cloth. The enamel specimens will then be rinsed under a steady stream of dw for three minutes, sonicated for three minutes in 2% microliquid soap and then rinsed again with dw for three minutes. Resulting specimens will have a thickness range of 1.8 to 2.2 mm. The specimen will have a minimum polished surface of 3 mm × 3 mm in the center of the enamel surface.

Figure 2
4 mm Round Human Enamel Specimen



4.2 Lesion Creation

The round enamel specimens will be partially demineralized using a modification of the method described by White [1987]. The human enamel specimens will be immersed for 24 hours at 37° C under static conditions in 40 ml of an acid buffer (0.05 mol/L lactate), 50% saturated with respect to hydroxyapatite and with 0.2% (wt/vol) carbopol 907 (BF Goodrich Co., USA) added (pH adjusted to 5.0 using KOH or NaOH), and then rinsed thoroughly with deionized water. The demineralized enamel specimens will then be stored in a moist environment to prevent dehydration.

4.3 6.3 Lesion Quality

Lesions will be inclined towards an overhead light until a reflection is obtained. Acceptable lesions with an intact surface-zone will have a continuous, uniform shiny surface, with no matt areas. Lesions with matt area(s) will be rejected. The following criteria will be used to select specimens for inclusion in the study:

- The lesioned areas of each specimen should be of equal and uniform opacity; and
- The lesioned areas of each specimen should possess a surface shine when exposed to light, thereby indicating an intact surface.

4.4 Efficacy Measurements and Evaluations

4.4.1 Surface Microhardness

The SMH test will be used to assess changes in the mineral status of partially demineralized enamel specimens. SMH will be measured using a Wilson 2100 Hardness Tester. Each enamel specimen will be secured on a 1-inch square acrylic block with sticky wax and then placed on the microhardness tester. Five baseline indentations spaced 100 µm apart will be placed with a Knoop diamond under a 50 gram load in the center of a flattened, polished sound enamel specimen. SMH will be determined by measuring the length of the indentations using Wilson 2100 - Clemex CMT Software (version 6.0.011). For enamel specimens to be acceptable for use in the study, the mean of the five baseline indentation length must be 43 µm with a standard deviation of ≤ 3µm.

After in vitro demineralization, the enamel specimens will be again SMH tested by placing five indentations 100 µm to the left of the baseline indentations (see Figure 2). To qualify for the study, the mean (n = 5) indentation lengths of the partially demineralized specimens must fall within a range of 100-140 µm with a standard deviation of ≤ 10 µm. After 7, 14 and 21 days of intra-oral exposure the enamel specimens will be again SMH-tested by placing five indentations 100 µm to the right of the baseline indentations (see Figure 2). The extent of remineralization will be calculated based on the method of [Gelhard et al., 1979].

$$\%\text{SMH recovery} = (D1-R)/(D1-B) \times 100$$

B = indentation length (µm) of sound enamel specimen at baseline
D1 = indentation length (µm) after in vitro demineralization
R = indentation length (µm) after intra-oral exposure.

Additionally, the absolute remineralization change will be computed as: SMH change= D1-R

4.4.2 Enamel Fluoride Uptake

The microdrill enamel biopsy technique as described by [Sakkab et al., 1984] will be used to analyze the fluoride content of the partially demineralized enamel specimens. Each enamel specimen will be mounted perpendicular to the long axis of a drill bit attached to a specially designed microdrill, and drilled to a depth of ~100 µm through the entire lesion (four cores per specimen; see Figure 2).
The drilling and sample collection will be performed in a static-controlled atmosphere to prevent loss of enamel powder due to charging effects. The enamel powder sample, pooled from the four drilling samples, will be transferred to an analyzer cup cap. 20 microliters (µl) of 0.5 Molar (M) Perchloric Acid ($HClO_4$) will be added to the enamel powder and the cap gently swirled to dissolve the powder. To the analyzer cap containing the 20 µl of $HClO_4$/enamel powder, 40 µl of citrate/ ethylene-diamine-tetraacetic acid (EDTA) buffer and 40 µl of de-ionized water will be added and immediately analyzed for fluoride

content using a fluoride specific electrode and pH/Ion meter. The diameter of the drill hole will be determined using a calibrated microscope interfaced with an image analysis system. The amount of fluoride-uptake by enamel will be calculated based on the amount of fluoride divided by the area of the enamel cores and expressed as µg F/cm^2.

4.5 Laboratory Data

As each specimen is analyzed, individual Excel files will be generated and imported into a validated relational database management system. After the analyses are completed, all enamel specimen surface microhardness, and enamel fluoride uptake data will be reviewed by the principal investigator before secure transfer to the sponsor. The investigator will document this review and documentation will be filed with the laboratory study files.

4.6 Specimen Retention

Laboratory specimens will be retained by the study site for twelve months following database lock.

5. STATISTICAL PLAN

5.1 Statistical Methods

Efficacy Analyses

The mean % SMH recovery and SMH change will be calculated using the five sets of indentations within each enamel specimen. The mean fluoride uptake will be calculated using the four samples within each enamel specimen. The mean % SMH recovery, SMH change and mean fluoride uptake will be computed for each enamel specimen at the different time points (1 specimen for 7 and 14 days and 2 specimens for 21 days) within a subject; if a subject is missing an enamel specimen for the 21-day reading, the single available enamel specimen will be used. The treatments will be compared using analysis of variance models (ANOVA) or analysis of covariance (ANCOVA) suitable for a crossover study.

To assess for potential carryover effects on the primary endpoint, the models will include random effects for subject and fixed effects for study period, product and carryover term. If the carryover term is not significant at the 0.1 level ($p>0.1$) then the final crossover model will not include the carryover term.

A repeated measures crossover model will also be carried out to explore the treatment time effect. This model will include a random effect for subject and fixed effects for study period, product, treatment times (7, 14 and 21 days) and treatment time by product interaction . For SMH change, B (indentation length (µm) of sound enamel specimen at baseline) or D1 (indention length (µm) after challenge) will be added as a covariate in the model. Additionally, the product by covariate interaction will also be assessed in this model. A 10% significance level will be used for all product comparison tests. No alpha-level adjustments for multiple comparisons adjustments will be applied.

If the data does not satisfy the normality criterion, log transformation or analogous nonparametric methods will be employed. Additional statistical techniques may also be applied in order to more fully understand the data.

The SnF_2 product used in Period 5 will be compared to the negative control treatment used in other periods using a paired t-test.

Safety Analyses

Adverse events reported during the study will be listed in the final report.

5.2 Hypotheses

The following hypothesis will be tested for each index and each time point:

Null: there is no difference between the treatments using pastes with different fluoride level.

Alternative: there is a difference between at least two treatments with different fluoride level.

Period 5 Hypotheses:

Null: there is no difference between SnF_2 and the negative control.

Alternative: there is a difference between SnF_2 and the negative control.

5.3 Number of Subjects

The number of subjects (16) was chosen for logistical reasons for this study.

5.4 Selection of Subjects

5.4.1 Intent-to Treat (ITT) Population

The subjects will be selected from the OHRI's IRB approved database of persons previously accepted into the partial denture panel (IRB #1110007150). Potential subjects will be screened to determine eligibility to participate in this study. Sixteen adult subjects, between the ages of 18 and 85 years, will be accepted and randomized in the study so at least 10 subjects can complete the study. The ITT population will include those subjects randomized and who had at least one exposure to product.

5.4.2 Per Protocol (PP) Population

The PP population will be a subset from the ITT population without any major protocol deviations.

6. DATA MANAGEMENT PLAN

The following steps will be taken to ensure the accuracy, consistency, completeness, and reliability of the data:

- documenting Clinical Trial Risk Assessment and Risk Management Plan;
- site initiation meeting;
- routine site and/or data monitoring;
- ECRF review against source documents;
- data management quality control checks;
- statistical quality control (QC) checks;
- continuous data acquisition and cleaning;
- internal review of data.

In addition, a Sponsor representative may conduct periodic quality assurance audits of the study processes, including, but not limited to, clinical site visits, laboratories, vendors and contract research organizations, the study database, and P&G's final study report. Data monitoring will ensure quality of collected information by detection of inconsistent and missing information. The investigational site will follow up on any queries from P&G Data Manager. If a query arises from a subject that was lost to follow-up, the site will take action to contact the subject and document any attempts made. The study monitor will ensure that the study was conducted in accordance with the protocol and any amendments, good epidemiological practice, and applicable laws and regulations.

Case Report Forms

The Data Manager will supply the paper and/or electronic CRFs to be used in this study. It is the responsibility of the Investigator to maintain and submit accurate and timely CRFs to the Sponsor. All hard copy CRFs will be filled out legibly in ink.

All questions should be answered. For paper CRFs, if an entry requires correction, a single line will be placed through the entry so as not to obscure the original record, the corrected entry will be initialed and dated by the individual making the change, and a reason will be given for the change. There will be no whiteouts or erasures. For electronic CRFs, if an entry requires correction, the change is made directly to the CRF in the database, the user is prompted to provide a reason for the change, and the correction is logged in by an electronic audit trail.

As necessary, the Data Manager may make specified allowable changes to the database without issuing a query to the site, as agreed upon by study site per this protocol. Examples of allowable changes include incorrect date formats, incorrect current year recorded (as in the start of a new year), and unambiguous spelling errors. Changes to common abbreviations and symbols to equivalent text to meet system or coding constraints (e.g., @ = at, ~ = approximately), may also be allowable. Values that are ambiguous or open to interpretation will be queried to the sites. It is the responsibility of the Data Manager to ensure all changes are supported by information contained elsewhere and/or are unambiguous.

6.1 Access to Source Data/Documents

The Investigator has the responsibility for ensuring that all source documents (i.e., study and/or medical records) and CRFs are completed and maintained according to the study protocol and are available at the site. Any CRF used as a source document must be identified as such in the Investigator Notebook.

6.2 Data Handling and Recordkeeping

The Investigator must retain the subject identification codes, informed consent documentation, clinical materials inventory, CRFs (paper or electronic media), medical records and other source data for a minimum of 2 years after the last regulatory approval has been received or the discontinuation of the study. The Investigator must receive written authorization from the Sponsor before destroying any study document. The Investigator will make the records available for inspection and copying upon the request of an authorized employee of a government authority or the Sponsor, at reasonable times. In the event the Investigator retires, relocates, or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records may be transferred to another person who will accept responsibility for the records. Notice of such a transfer must be given in writing to the Sponsor.

The subject will be identified with a unique identification code assigned by the Principal Investigator to each trial subject to protect privacy. The identification codes are used in lieu of the subject's name when the Principal Investigator reports all adverse events and other trial related data. These codes will be used on all study documents for the subject's confidentiality, as stated in section 2.11 of the ICH-GCP.

Any advertisements used in recruitment of subjects must receive prior approval from P&G and/or the Investigator's IRB. A copy of the IRB-approved advertising and the documentation thereof must be provided to P&G.

Following completion of the study, the Investigator shall submit a final report to the Sponsor describing the conduct of the study, deviations from planned conduct, early withdrawals and subject accountability, adverse events, and other information on study conduct. The Investigator's IRB may require more frequent status reports.

7. ETHICS

Subject participation is voluntary. Subjects have a right to refuse participation in the research study at any stage. The study will be conducted in accordance with all international laws and regulations, and also national laws and regulations, and in accordance with any applicable guidelines. Informed consent will be obtained from potential participants of the study. Beforehand the study site should fully inform the subject regarding all the aspects of the clinical study, including objectives, collected data, expected risks and benefits of participation, and voluntary participation in the study.

Conduction of the study will comply with the principles of ICH-GCP. Before study start an approval of Independent ethical committee for conduction of the present study will be obtained. A list of investigators and investigational sites will be approved. All documents and data related to the study are strictly confidential. Protocol contents cannot be disclosed to third parties without written permission of the Sponsor.

Sponsor of the study is obliged to adhere to confidentiality of the study participants' personal data. Any information identifying the subject's identity should not be disclosed. The necessary personal data of the subjects as part of the study (for example, gender, age) will be collected only for achieving study purposes and in minimum quantity. The study site will maintain an identification list of all screened and enrolled subjects. Therefore, names and surnames of subjects will not be disclosed or reported to the Sponsor. If subject name and surname are mentioned in any document, then before sending a copy of such document to the Sponsor these data will be erased. Results of the study stored in electronic format will be kept in accordance with current information protection laws. Sponsor employees or regulatory authorities' representatives should not contact the subject directly. Before enrollment into the study, subjects will be informed regarding confidentiality provisions and use of their personal data, including necessity of access to them of the monitor and other authorized people (in case of auditing, inspection and etc.).

8. FINANCING AND INSURANCE

Financing and insurance can be found in signed and executed clinical study agreement.

9. PUBLICATION POLICY

The results of this study may be published in oral presentations, written abstracts, written manuscripts, and/or with regulatory agency.

APPENDIX

STUDY RESTRICTIONS:

- During the study, do not use any oral hygiene products other than the products given to you unless you have been told so by the study staff. You may use floss if this is your normal practice.
- Your lower partial denture and upper partial or full denture, if applicable, may be cleaned outside of your mouth with water only.
- Do not use any denture cleaning products.
- You may remove your lower partial denture for short periods (to rinse your mouth with water after meals and snacks), however, you will have to wear it 24 hours a day, every day, including when you sleep, during each two-week test period.
- Do not use any denture adhesive on your lower partial denture. If you need an adhesive for your upper denture, you must use a zinc free adhesive like Poligrip®.
- Please refrain from eating canned sardines during the course of the study.
- Please refrain from having hard candy or nuts when the enamel specimens are in place.

IMPORTANT TO REMEMBER

- Record any changes in your health, medications (prescription and over the counter medications) or treatments.
- Bring your diary and remaining product tubes to your next study visit.

WASHOUT PERIOD INSTRUCTIONS

1) Remove your lower partial denture and upper partial or full denture, if applicable.
2) Brush your natural teeth with provided wash out toothpaste and toothbrush.
3) Brush your lower partial/denture with water only.
4) Place the partial/denture back in your mouth.
5) Please remove your partial denture at night.

TEST PERIOD INSTRUCTIONS

PRE-STUDY PRODUCT USE INSTRUCTIONS (in the morning after breakfast and at night just before bedtime)

Because you are brushing your teeth with your lower partial denture in place, it is important that you take care of your partial denture and your natural teeth in the places that might be missed during study product brushing. The following instructions should be followed **before** brushing with study product:

1) Remove your lower partial denture and upper partial or full denture, if applicable.
2) Brush your natural teeth with water only, paying close attention to the teeth surfaces that might be covered by the partial/denture.
3) Brush your lower partial/denture with water only. Be sure to brush the areas that can't be reached when the partial is in your mouth. DO NOT BRUSH THE SPECIMEN SITES.
4) Place the partial/denture back in your mouth and follow the instructions below for study product use.

STUDY PRODUCT USE INSTRUCTIONS

5) Put a strip of toothpaste on the toothbrush, covering the full head of the brush.

6) Set your timer for 1 minute.

7) Measure 15mL of tap water into the dosing cup provided.

8) Brush the biting surfaces of your back teeth in all four quadrants of your mouth (do not brush the specimen sites) for a total of one timed minute and then spit out slurry.

9) Rinse your mouth with the 15mL of measured water for 10 seconds and spit out.

10) Record each brushing in the diary.

HOME USE DIARY

a) **Shaded area shows you an example of how to complete your diary.**
b) **Make an entry for each day of the study starting with the first brushing.**
c) **Tick the box each time you brush.**
d) **Add a comment if you have not brushed according to the instructions provided.**

Treatment Day	Date	Brushing Time AM / PM	Brushed with Full Ribbon of Toothpaste for 1 Minute	Spit Out Slurry	Rinsed with 15 ml Tap Water for 10 Seconds
0	01/12/2021	8:00am	✓	✓	✓
		10:00pm	✓	✓	✓
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

11					

Treatment Day	Date	Brushing Time AM / PM	Brushed with Full Ribbon of Toothpaste for 1 Minute	Spit Out Slurry	Rinsed with 15 ml Tap Water for 10 Seconds
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					

Date	Comments related to changes in your health, medications or brushing procedures

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