Evaluation of the efficacy and safety of a loop stretchable dental floss in controlling gingivitis and supragingival plaque removal during thirty days

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Amendment 2

Support Provided by:
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Abbreviations (Table 1)

AE	Adverse Event
BOMP	Bleeding on Marginal Probing
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDA	Food and Drug Administration Amendments Act of 2007
GCP	Good Clinical Practice
MGI	Modified Gingival Index
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IRB	Institutional Review Board
NCT	National Clinical Trial
NSAID	Non-Steroidal Anti-Inflammatory Drug
NIH	National Institutes of Health
OH	Oral Hygiene
PLI	Plaque Index
PTFE	Polytetrafluoroethylene
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TNF-AB	Tumor Necrosis Factor Alpha Blocker
UHMPE	Ultra-high-molecular-weight polyethylene
UP	Unanticipated Problem
US	United States

1.0 Background & Rationale

Interproximal plaque control is the most efficient method to prevent inflammation at the papillary gingival unit. [1] This type of cleaning cannot be achieved with tooth brushing alone; flossing needs to be incorporated into the oral hygiene procedures to achieve and maintain the health of the interdental papilla. [2] Over the years, different types of materials have been used to produce dental floss, including nylon,[3] PTFE, silk, and UHMUPE, among others,[4]. Even though all these dental flosses are effective in removing dental plaque,[5] the users' subjective preference is based on the surface roughness and tensile strength. [4] The user's preferences regarding floss handling also have to be considered; loop floss provides a new design that appears to be easy to use and improves the user's manual dexterity. [6, 7] This study aims to evaluate the efficacy and safety of a stretchable floss controlling gingivitis and supragingival plaque removal between control and experimental groups in thirty days.

2.0 Objective(s)

2.1 Primary Objective

This study aims to evaluate the efficacy and safety of Floss Loops stretchable floss for controlling gingivitis and supragingival plaque removal in a control and an experimental group over thirty days.

3.0 Outcome Measures/Endpoints

3.1 Efficacy Outcome Measures

There will be two efficacy indexes used in this study; each index will be an overall total dentition score:

A. **For Gingivitis Assessment**: The Lobene modified gingival index (MGI) will be used. This is a non-invasive index that assesses the health of the gums based on their visual clinical appearance. (Table 2) Each tooth will be evaluated visually on six surfaces (Mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual). Each surface will be scored based on the inflammatory visual changes, severity, and extent. For the MGI calculation, all the values for each tooth will be added and divided by the number of surfaces examined. A score of 0 will represent healthy gingiva, a score of 1 and 2 will represent mild inflammation, a score of 3 will represent moderate inflammation and a score of 4 will represent severe inflammation. [8] Additionally, a similar calculation for the interproximal surfaces MGI score will be performed using only interproximal surfaces from each tooth.

B. **Bleeding on Marginal Probing (BOMP)**: Bleeding on probing will be assessed at six surfaces (Mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual). A periodontal probe with a rounded tip 0.5mm in diameter will be gently inserted into the gingival crevice to a depth of approximately 2 mm and run at an angle of approximately 60° about the longitudinal axis of the tooth, except for third molars if present. BOMP will be read up to 30 seconds after probing. The surfaces would be scored as negative

(0=no BOMP) or positive (1=BOMP). The percentage of bleeding sites will be determined by adding up the number of bleeding sites, dividing this number by the total number of probing sites, and multiplying by 100. [9, 10] Additionally, a similar calculation for the interproximal surfaces BOMP score will be performed using only interproximal surfaces from each tooth.

Table 2: Lobene Modified Gingival Index (MGI)

Score	Inflammation	Description
0	**Normal**	Normal gingiva
1	**Mild inflammation**	Slight changes in color and texture, but no in all portions of the gingival marginal or papillary
2	**Mild Inflammation**	Slight changes in color and texture in all portions of the gingival marginal or papillary
3	**Moderate**	Bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary
4	**Severe Inflammation**	Erythema, edema, and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion, or ulceration.

C. **For the Dental Plaque Assessment:** Dental plaque will be identified using a disclosing solution and scored using the Turesky modification of the Quigley and Hein Plaque Index (PLI). (Table 3) Each tooth, except the third molars, will be scored on six sites (Mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual). The plaque score index for an individual is determined by adding all the individual scores and dividing the total score by the number of surfaces examined. [11] Additionally, a similar calculation for the interproximal surfaces PLI score will be performed using only interproximal surfaces from each tooth.

Table 3: Quigley-Hein Plaque Index Scores with Turesky Modifications (PLI)

Score	Description
0	No plaque
1	Separate flecks of plaque at the cervical margin of the tooth
2	A thin continuous band of plaque (up to one mm) at the cervical margin of the tooth
3	A band of plaque wider than one mm but covering less than one-third of the crown of the tooth
4	Plaque covering at least one-third but less than two-thirds of the crown of the tooth
5	Plaque covering two-thirds or more of the crown of the tooth

3.2 Safety Outcome Measure

A. Safety outcomes will be measured by assessing any study-related soft or hard tissue abnormalities. Oral soft tissue examinations will be assessed at the baseline and the end of study. Any abnormal oral findings will be recorded and evaluated to assess causality and possible relation to the study product. Abnormal findings during the study will be recorded as an AE or SAE.

4.0 Eligibility Criteria

4.1 Inclusion Criteria

- Adult subjects between 18 to 65 years old
- Willing to read and sign the IRB-approved informed consent
- Healthy, as determined by pertinent medical history at the examiner dentist's discretion
- A minimum of 20 natural teeth (excluding third molars) with at least two scorable surfaces per tooth (teeth with full crowns, large/extensive restorations on the interproximal areas, and orthodontic bands will not be included in the tooth count)
- Mild to moderate plaque and gingivitis MGI of 1.75 or greater (Based on the Lobene-modified gingival index score)[9]. This calculation will be made based on a whole mouth score
- PLI of 1.95 or greater (Based on the Turesky modification of the Quigley and Hein Plaque Index) calculation based on a whole mouth plaque score
- Be willing to comply with study visits and requirements

4.2 Exclusion Criteria

- Presence of any acute or chronic condition, organ system disease, or medication that, in the principal investigator's opinion, could compromise the subjects' ability to participate in the study
- Gross oral pathologies, including caries, calculus, or soft tissue conditions that show evidence of chronic neglect
- Orthodontic appliances (except for fixed lingual retainers, teeth will be excluded from the teeth count) or any removable prosthesis
- Evidence of acute periodontal conditions or periodontitis with pockets greater than 5 mm on more than one site
- Taking antibiotics two weeks before baseline procedures and throughout the study
- Need for antibiotic prophylaxis prior to dental procedures
- Use of daily anti-inflammatory drugs (NSAID, TNF- AB, others) within 30 days before baseline
- Pregnant, wanting to get pregnant, or breast-feeding female
- Acute Temporomandibular Disorders (TMD)
- Subject who has participated in other studies (including non-medicinal studies) involving product(s) within 30 days before study entry
- Subject who has previously been randomized in this study
- Self-reported allergy to disclosing solution ingredients (red dye #28)
- An employee of the study site directly involved with the study

5.0 Study Design

This will be a single-blind, single-center, parallel, randomized controlled clinical trial to evaluate the efficacy and safety of Floss Loops stretchable floss for the control of gingivitis and supragingival plaque removal between two groups, control (no-floss) and experimental (flossing with Floss Loops), during thirty (+/- 2 days). A flowchart of the study is shown in Fig 1

Subjects will be asked to refrain from oral hygiene (OH) procedures the evening before and the morning of the screening visit, chewing gum for 12 hours and eating or drinking anything 2 hours before oral assessments. During the study, subjects will be asked to clean their teeth twice daily using only the toothbrush provided and an assigned ADA-accepted toothpaste. They will also be asked to abstain from professional dental cleaning during the study. They will also need to refrain from elective dental procedures during the study.

At the screening/baseline visit, potential subjects will be given the IRB-approved informed consent form to read and ask questions, and adequate time to decide about their participation will be provided. A study representative trained and delegated by the Principal Investigator will review the IRB-approved consent and answer any questions the potential subject might have before the subject signs/dates the consent. After the subject signs and dates the consent, the study representative will sign and date the consent to confirm that the consent process was completed before initiating any study procedures. The subject will be given a copy of the signed consent.

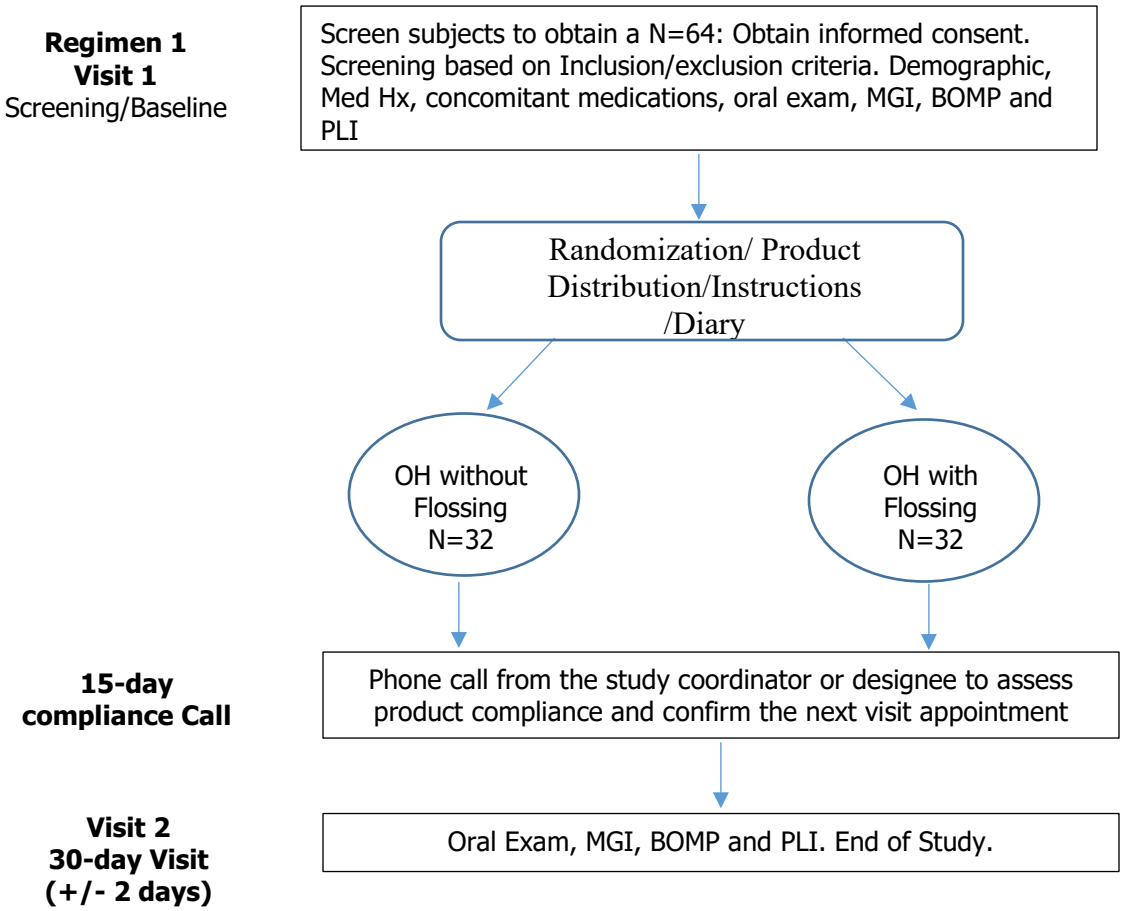
Information related to the subject's demographic, medical, and concomitant medications will be collected and review. An oral examination and MGI, BOMP and PLI assessments will be performed by assessing the subject's whole mouth gingival conditions and plaque accumulation. Each outcome measurement score will be given, and subjects must have a Modified Gingival Index (MGI) of 1.75 or greater and a Plaque Index (PLI) of 1.95 or greater to comply with the study inclusion criteria. Other dental characteristics specified on the inclusion/ exclusion criteria will be assessed to determine subject eligibility.

A total of 64 subjects who meet the study criteria will be enrolled and randomized into two groups. 32 subjects will be assigned to the control group (Brushing twice a day only and no flossing) and 32 to the experimental group (Brushing and Flossing with the stretchable loop floss at least twice daily). Each subject will be instructed to clean their teeth daily using only the provided toothbrush with an assigned ADA-accepted fluoride dentifrice. Subjects assigned to the control group will brush their teeth at least twice a day only, and subjects in the experimental group will brush and floss with the provided stretchable loop floss at least twice a day. To assess compliance, each subject will be asked to fill out a diary every time they brush/floss. On day 15 (+/- 2 days) of product use, subjects will receive a phone call from the study coordinator or designee to assess brushing/flossing compliance. At day 30 (+/- 2 days), study hygiene and gum chewing restrictions as well as eating restrictions will apply, and the efficacy assessments will be repeated, subjects will be dismissed, and the study will finish.

All clinical examinations will be performed by examiners who will be blind to the subject's oral hygiene procedures.

The sponsor (Loops, LLC) will provide all the study materials, including the stretchable loop floss, toothbrushes, and toothpaste, before the beginning of the study. The stretchable loop floss is packaged with 30-33 floss in a clear bag along with the instructions for use.

Fig 1 Study Flow Diagram



6.0 Enrollment/Randomization

Recruitment of potential subjects will be using the following methods:

- Using fliers in the Indiana University School of Dentistry and other Indiana University Indianapolis campus locations, including IU Health facilities. Fliers may also be posted on Facebook.

- Sending outreach email messages to subject volunteers who are part of the All IN for Health Volunteer Registry using All IN For Health and iConnect.
- Screening subjects from the Electronic Dental Records at IUSD. Potentially eligible subjects' names and addresses must be obtained from the patient's electronic dental record system at IUSD. The provider sending the letter will be the Associate Dean of Clinical Affairs, Office of Clinical Affairs, at IUSD, who provides administrative oversight of IUSD clinics. The recruitment letter will be submitted to the IRB as a recruitment tool.
- Sending flyers and/or calling subjects who previously participated in other Oral Health Research Institute studies. (OHRI)

Interested individuals can leave their contact information at the OHRI recruitment line or the OHRI recruiting email address. The potential subjects will be contacted, and a team member will conduct a screening phone interview. If the potential subjects appear to qualify, they will be scheduled for the screening/baseline visit.

A sufficient number of subjects will be screened to participate, and 64 will be randomized. We intend to randomize a nearly equal number of female and male subjects. A unique screening number will identify all subjects screened for study participation. Screening numbers will be assigned according to appearance at the study site. Subjects who meet all inclusion and exclusion criteria will be randomized into the study. Block randomization will be used to assign subjects to one of the two sequences: control regiment or experimental regimen. Randomization will be stratified by gender at birth. Randomization numbers will be assigned using randomization schedules provided by the IU Department of Biostatistics and Health Data Science statistician.

The clinical examiner will remain blind to the individual subjects' treatment group during the entire study. If the treatment assignment must be unblinded for a subject, such as in the case of an emergency, the Investigator will provide written documentation of the unblinding request. Unblinding would involve withdrawing the subject from the study. Otherwise, blinding will not be broken, unless required in the circumstances detailed above, until all subjects have completed the final study visit and the database has been monitored, locked, and approved by the Investigator.

7.0 Study Procedures

7.1 Visit 1: Screening/Baseline Visit

Following the consenting procedure and demographic/medical history data collection, the following will be performed:

a. An oral exam will be completed, and MGI, BOMP and PLI will be assessed and recorded.
b. Inclusion/Exclusion criteria will be reviewed to determine eligibility.
c. If qualified, the subject will be randomized to an experimental group.
    i. A qualified study team member will prepare and distribute the randomized study product, toothpaste, how-to-use instructions (Append 1), lifestyle restrictions (Append 2), and compliance dairy to the subject. Subjects will be asked to brush their teeth twice daily with the toothpaste and toothbrush provided.

ii. Subjects in the experimental group must use the provided stretchable dental floss at least twice a day after brushing their teeth.
iii. Subjects will be asked to refrain from their usual oral hygiene procedures and professional dental cleaning during the study. They will also need to refrain from elective dental procedures.

d. Each subject will be scheduled for a telephone call on Day 15 (+/—2 days) and an appointment on Day 30 (+/—2 days).
e. Subject payment will be given.

7.2 15-day Compliance Call

a. On day 15 (+/—2 days), a staff member will call subjects to assess product compliance.
b. During this call, subjects will be asked if they have any issues using the provided products and will be reminded to record the requested information in the provided diary.
c. They will also be reminded of the appointment date and time for the 30-day visit at the study site.
d. Subjects will be compensated for the phone call.

7.3 Visit 2: 30-day visit

a. On day 30 (+/—2 days), subject continuance criteria will be assessed.
b. Randomized products will be collected.
c. An oral exam will be completed, and MGI, BOMP and PLI will be assessed.
d. Subject payment will be given.

7.4 Oral Exam

The study dentist will complete an oral soft and hard tissue (OSHT) examination at screening/baseline and each study visit. The exams will be conducted via a visual examination of the oral cavity and perioral area utilizing a light source, dental mirror, gauze, 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, tongue, sublingual area/floor of the 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, residual roots, faulty restorations, and implants. Observations will be listed as "Absent" or "Present," and conditions noted as present will be described.

7.5 Modified Gingival Index (MGI)

The Lobene-modified gingival index (MGI) will be used. This is a non-invasive index that assesses the health of the gums based on their visual clinical appearance. (Table 2) Each tooth will be evaluated visually on six surfaces (Mesio-buccal, buccal, distobuccal, mesio-lingual, lingual, and disto-lingual). Each surface will be scored based on the inflammatory visual changes, severity, and extent. A score of 0 will

represent normal. A score of 1 and 2 will represent mild inflammation, a score of 3 indicates moderate inflammation and a score of 4 indicates severe inflammation). (8) A single, trained examiner will complete all the examinations. For the whole-mouth MGI calculation, all the values for each tooth will be added and divided by the number of surfaces examined. A similar calculation for the interproximal surfaces MGI score will be performed using only interproximal surfaces from each tooth.

7.6 Bleeding on Marginal Probing (BOMP)

Bleeding on probing will be assessed at six surfaces (Mesio-buccal, buccal, distobuccal, mesio-lingual, lingual, and disto-lingual). A periodontal probe with a rounded tip 0.5mm in diameter will be gently inserted into the gingival crevice to a depth of approximately 2 mm and run at an angle of roughly 60° about the longitudinal axis of the tooth, except for third molars if present. BOMP will be read up to 30 seconds after probing. The surfaces would be scored as negative (0=no BOMP) or positive (1=BOMP). The whole-mouth BOMP score will be calculated as the percentage of bleeding sites, determined by adding up the number of bleeding sites, dividing this number by the total number of probing sites, and multiplying by 100. [9] A similar calculation for the interproximal surfaces BOMP score will be performed using only interproximal surfaces from each tooth.

7.7 Plaque Index (PLI)

Supragingival plaque will be assessed following disclosure with dye solution on the facial (buccal) and lingual surfaces of all scorable teeth according to the criteria of the Turesky modification of the Quigley-Hein Plaque Index (Table 3). Each tooth will be visually assessed on six surfaces (Mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual). For PLI calculation, all the individual scores will be added and divided by the number of surfaces examined for calculation of the whole-mouth PLI score. A similar calculation for the interproximal surfaces PLI score will be performed using only interproximal surfaces from each tooth. The same trained examiner will also perform this assessment.

7.8 Home Use Diary

At the start of the study period, subjects will be provided with a diary to record the date and the time of the morning (a.m.) and evening (p.m.) brushing/flossing procedures and any deviation from the brushing regimen. In addition, subjects will record any new or any changes in pre-existing medical conditions, medications, or treatments or any change in signs or symptoms.

Subjects must bring the completed diary to the end of the treatment visit. Study staff will review the diary with the subject to confirm treatment compliance and clarify listed medical conditions, medications, and treatments. Subjects will be allowed to miss up to 10% of their flossing times (6 times). Those subjects who miss more than 6 flossing times will be considered non-compliant.

7.9 Loops Stretchable Floss ™

Floss Loops is a stretchable dental floss made of polyurethane. It has a half circumference of 55.0 mm, a thickness of 0.3 mm, and a cut width of 2.0 mm. It has a two-color presentation, and each color represents a different flavor. The blue color is mint, and the clear, semi-transparent is antiseptic. For this study, we will be using the mint loops blue color. It is packed in a transparent, easy-to-open, Ziplock plastic bag with 30 to 33 units (Package by weight count). This floss has been primarily designed for use by inmates and patients where safety is the principal concern, and it has been used by Federal, State, and County Correctional Facilities since 1996.

8 Reportable Events

Adverse events are not anticipated with Loops Stretchable Floss or its packaging. The product is designed to break easily if too much pressure is applied. No anticipated gum lacerations are expected. However, any tissue changes during the study will be documented and reported. The product is packaged in a small clear plastic bag that is easy to open and close.

Subjects will be questioned regarding any general health or oral complaints and symptoms they have experienced during or following their treatment. Any findings will be documented on the AE CRF. Subjects reporting AEs outside the scheduled clinical visit will be assessed by the study dentist and/or principal investigator as soon as possible.
All AEs, regardless of severity or relationship to the test product, will be recorded.

Serious AEs include any events resulting in death, decreased life expectancy, life-threatening situations, persistent or permanent disability/incapacity, hospitalization, or congenital anomaly/congenital disability. Within 24 hours, the Investigator will submit a written report documenting the circumstances of the serious AE. The IRB will be notified within five days of the incident.

9 Data Safety Monitoring

The PI and the entire study team will monitor the data and safety. They will monitor data quality, subject recruitment, accrual, retention, outcome, and adverse event data, or assessment of scientific reports, results of related studies that may impact subject safety, and procedures designed to protect subjects' privacy. Safety data will be monitored regularly and immediately upon discovery of any SAE, major study event, or protocol deviation.

10 Study Withdrawal/Discontinuation

Subjects may withdraw from the study for any reason and at any time during the study without penalty. Their decision to stop study-related procedures will not affect their dental treatment. The Principal Investigator may also withdraw the subject if they feel study participation is a safety concern or the subject is not compliant with study procedures.

11 Statistical Considerations

MGI, BOMP, and PLI whole-mouth and interproximal surface scores will be summarized at baseline and 30 days for each group: sample size, mean, standard deviation, standard error, 95% confidence interval for the mean, 25th percentile, 50th percentile, 75th percentile, minimum, maximum. Whole-mouth and interproximal surface MGI, BOMP and PLI change from baseline to 30 days will be calculated for each subject and will be summarized as above. Comparisons between the experimental and control groups for differences in baseline MGI, BOMP and PLI whole-mouth and interproximal surface scores will be performed using two-sample t-tests. Comparisons between the experimental and control groups for differences in 30-day MGI, BOMP and PLI whole-mouth and interproximal surface scores will be performed using two-sample t-tests. 95% confidence intervals for differences between groups will be calculated for all comparisons. For all analyses, the distribution of the MGI, BOMP and PLI whole-mouth and interproximal surface scores will be examined, and a transformation of the data (e.g. natural logarithm) prior to performing the two-sample t-tests or nonparametric Wilcoxon Rank Sum tests will be used if necessary. If data are normally distributed but variances for the two groups are different, the two-sample t-test will incorporate heterogenous variances. A two-sided 5% significance level will be used for all tests. To demonstrate efficacy at 30 days, the experimental regimen must be statistically significantly lower than the control regimen with a mean difference of at least 10% for MGI and must be statistically significantly lower than the control regimen for PLI.

Oral soft and hard tissue examination results for each structure/type will be summarized at baseline and 30 days for each group: total sample size, frequency and percent abnormal, frequency and percent normal. Comparisons between the two groups for differences in 30-day OST percent abnormal for each structure/type will be performed using chi-square tests, or Fisher's Exact tests if the sample sizes in the contingency table are less than five. A two-sided 5% significance level will be used for all tests.

Sample size calculations are based on a prior study (Bosma 2024). The MGI mean in the control group at 4 weeks was 2.73, with standard deviation 0.35. The study will need to detect a 10% improvement in the experimental group compared to the control, so this study will be powered to detect a MGI difference of 0.27 between groups. With 80% power and two-sided 5% significance level in a two-sample t-test calculation, the study will need 28 subjects per group to complete the study. ADA guidelines require 30 subjects per group to complete the study. To account for dropout, 32 subjects will be enrolled per group.

12 Privacy/Confidentiality Issues

To maintain privacy, discussions about the study, the consenting process, and all other study procedures will be conducted in a dental office away from public places.

Paper records will be kept confidential by being stored in a locked area that only study personnel can access. Subjects will be given a study number that will identify all documents. Documents containing the subject's name (like the signed consent form) will be stored separately from other study documents.

Electronic data will be stored in an encrypted, password-protected computer file that only study personnel can access.

13 Follow-up and Record Retention

As per Indiana law, study records will be maintained for at least seven years.

14 References

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2. Shamsoddin, E., *Dental floss as an adjuvant of the toothbrush helps gingival health.* Evid Based Dent, 2022. **23**(3): p. 94-96.
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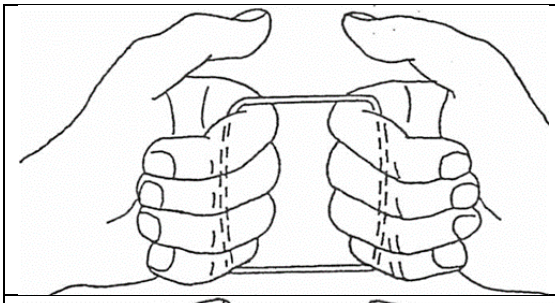
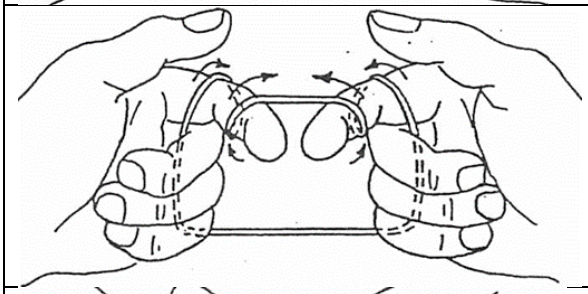
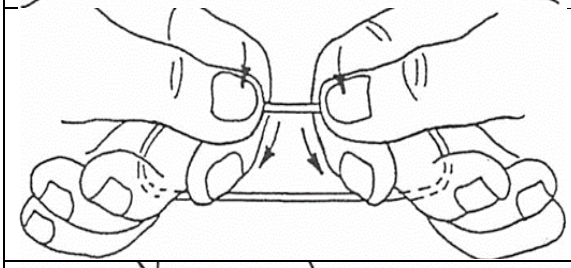
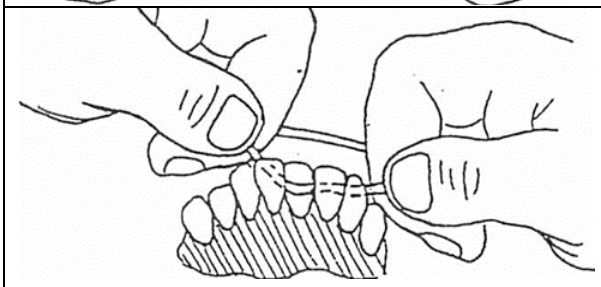
15 Appendix

1.0 Flossing Instructions

	Grasp the Floss Loops® in opposite hands and slightly stretch apart to create tension
	Wind the Floss Loop® once around the index finger of each hand
	Place thumbs on each forefinger over the Floss Loops® strand
	Insert Floss Loop® between teeth and slide along the side of each tooth to clean both above and below the gum line

2.0 Subject's Restrictions

During the study,

- Do not have your teeth professionally cleaned
- Refrain from elective dental procedures such as fillings
- Do not use any other oral hygiene product or procedures other than provided during your study visit

Prior to your next appointment:

- Do not brush or floss the evening before or the morning of your appointment
- Do not chew gum 12 hours before your appointment
- Do not eat or drink anything 2 hours before your appointment