Tufts UNIVERSITY | School of Dental Medicine

The effect of instructed dental flossing on interdental gingival bleeding: A randomized controlled clinical trial

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ABSTRACT

Aim and Hypothesis:

The main aim of this study was to test the effectiveness of using dental floss daily with specific instructions and with a fixed frequency interval. This is compared with subjects flossing without flossing instructions to determine the degree of gingival inflammation as evidenced by BoP.

A second aim was to compare the completeness of plaque removal of flossing by a dental professional with the completeness of a subject's self-flossing following instruction with the specific technique as evidenced by using disclosing pills at various time periods (initial and after 2 months).

The hypotheses were: 1) Instructed daily flossing for subjects who exhibit gingivitis reduces bleeding on probing (BoP) of interdental sites more than for subjects who are uninstructed and floss with their usual intervals. 2) Professional flossing reduces interdental plaque more effectively than the subjects flossing for themselves. 3) Instructed subjects can remove more interproximal plaque after practicing the techniques daily for 2 months than they could when initially instructed. 4) The instructed subjects and the clinician will be equally complete with plaque removal at the fourth clinical evaluation.

Materials and Methods:

The study was designed to be a two-group parallel, eight-week home use experiment. Thirty-six subjects diagnosed with gingivitis were recruited and assigned to one of two groups: Group A (Test) was given instructed dental flossing technique, Group B (Control) was not given any instructions for flossing and asked to floss in the manner and frequency that they normally do. At baseline, the interproximal plaque and bleeding on probing (BoP) measurements of each tooth were recorded. Patients returned for evaluation at two, four and eight week intervals. BoP and plaque were recorded at each visit. Group A subjects were observed for their flossing techniques and were reinstructed as needed. At baseline, for Group A, the clinician flossed the subject's teeth on one side (half) of the mouth. The study subject was instructed to observe the professional. Following this, the subject flossed the other side (half) of the mouth. A similar plaque score was determined. This was repeated for group A at Visit 4 and for Group B at Visit 4.

Results:

The instructed flossing group showed significantly greater reductions in BoP scores than the non-instructed control group at 8 weeks. Group A showed a mean reduction of 18.07% of sites while Group B showed a mean reduction of 6.44% of sites ($P = 0.034$). As for Group A plaque scores, there was no statistically significant difference in comparing Visit 1 vs Visit 4 in terms of the instructor's % plaque removal vs. the subjects' % plaque removal in analyzing how close the subjects' efficacy of flossing compared with the instructor's at baseline and Visit 4. The

instructor outperformed the subject by 9.30% at baseline while this difference dropped to 2.81% at Visit 4 (P = 0.147).

Conclusions

Instructed daily flossing with the floss adapted to the proximal surfaces of the teeth in a horizontal and vertical motion is more effective in the reduction of interproximal BoP compared with individuals flossing with no instructions and without a mandated daily interval. Not all subjects who were taught the flossing technique, accurately performed the technique upon first trial and required a learning phase. As for plaque reduction, the current study failed to show a clinically significant difference between an instructor performing the flossing technique with the subjects performing the same flossing technique. Both subjects and instructor were able to remove plaque with the described flossing technique in up to 50% of sites.

DEDICATION

To my parents, Neveen and Ashraf, who without there continuous help and support would not have made this possible

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADA: American Dental Association

BoP: Bleeding on Probing

CAL: Clinical Attachment Loss

CO-I: Co-Investigator

ICF: Informed Consent Form

IQR: Interquartile range

IRB: Internal Review Board

PD: Probing Depth

PI: Principal Investigator

SD: Standard deviation

TUSDM: Tufts University School of Dental Medicine

LIST OF SYMBOLS

® : Registered Trademark

™: Trademark

The effect of instructed dental flossing on interdental gingival bleeding: A randomized controlled clinical trial

I- Introduction

Systemic and gingival Inflammation:

In the 2017 workshop for developing a new classification for periodontal disease, when attempting to define gingival health, Lang et al mentioned the term “pristine clinical health” as the absence of clinical signs of inflammation, the absence of Bleeding on Probing (BoP), and probing depths no greater than 3mm. They also used the term “clinically healthy” to define patients with very low levels of clinical signs of inflammation and BoP [1]. The end goal among clinicians is helping patients to establish a clinically healthy state with reducing and when possible, eliminating clinical signs of inflammation and BoP.

Inflammation is the process by which the body responds to an injury or a stimulus. It is characterized by classical signs described in the first century AD, dolor (pain), calor (heat), rubor (redness), tumor (swelling) and functio laesa (loss of function)[2]. Inflammation is useful in controlling infection, promoting healing, and helping to return the affected part of the body to a state of homeostasis[3]. A stimulus can be either microbial or non-microbial in origin. During the process of acute inflammation, the blood vessels become dilated for the delivery of plasma cells and leukocytes to the site of infection/injury. Microbial stimuli in particular trigger toll-like receptors and NOD (nucleotide-binding oligomerization-domain protein)-like receptors that activate the innate immune response. Macrophages and mast cells are also present at the site and result in a release of multiple inflammatory mediators. These mediators cause a release of inflammatory exudates in tissue that help to clear the stimulus. A successful response results in elimination of the stimulus followed by a repair phase. Unresolved inflammation can leads to

inflammatory cellular damage, possible sepsis, and pathological systemic consequences [4]. It was also shown that local and systemic uncontrolled inflammation can have a role in tumor progression and affect the course of treatment and prognosis of certain cancers[5].

Gingival inflammation is not different from systemic inflammation. In fact, one can say that gingivitis is a systemic disease as it is a hematological event. It can occur in the form of a plaque induced or non-plaque induced disease entity. Non plaque induced gingival diseases are usually a manifestation of a systemic condition. They have been most recently classified into several categories including but not limited to genetic disorders, specific (non bacterial) infections, inflammatory lesions, reactive lesions, neoplasms, endocrine lesions, traumatic lesions, and gingival pigmentations[6]. Treatment usually involves treating the underlying condition for a definite resolution of the disease.

Microbial (bacterial) threat:

Plaque induced gingivitis is the most common form of gingivitis affecting a significant portion of the population. It is defined as "inflammation of the gingiva in the absence of clinical attachment loss" [7]. It is considered a bacterial induced inflammation. Dental biofilm and plaque accumulation over a set period of time was shown to play a major role and are established as direct etiologic factors in gingival inflammation[8]. Dental plaque is considered a multi-species biofilm. For dental plaque to mature it undergoes a numerous step process without any disruption. This process starts with the formation of a thin film also called conditioning film, which forms from the interaction of molecules with the tooth surface seconds after cleaning the tooth. The second step is a very weak reversible adhesion of some microbes which are found in

the saliva and gingival crevicular fluid. Various species are usually found sub-gingivally and supra-gingivally. Following that is a stronger adhesion process formed by the interaction between the surface molecules and the microbial surface adhesins[9], Next the, secondary and late colonizers begin to adhere by the same cell surface adhesins in a process termed co-adhesion. This is followed by multiplication of the bacteria and formation of a matrix scaffold that maintains the integrity of the biofilm.

Bacteria can benefit greatly when growing in a community[10]. Bacteria growing in dental plaque are shown to express different phenotypes than if the same species were grown planktonically. These multiple species are shown to be more tolerant to anti-bacterial agents when present in biofilm[11]. This can be explained by a process called quorum sensing in which a bacterial communication system is developed in the dental plaque complex[12]. In this oral biofilm, certain molecules are produced, which start to affect the gene expression of certain bacteria and allows them to better tolerate the surrounding environment.[13] An example for that is *P. gingivalis* which is a periodontal pathogen that was shown to have 18% difference in its genome when grown in a biofilm[14]. Other benefits of bacteria growing in a community as dental plaque is the ability of anaerobic bacteria to grow at later stages since early aerobes consume the oxygen content of the environment.

The association of dental plaque with gingival and periodontal diseases has led to multiple theories that explain the role in the pathogenesis of this disease process. The first theory was the *non-specific plaque hypothesis* and was based on the idea that bacterial accumulation adjacent to the gingival margin leads to gingival inflammation due to the secretion of the bacterial toxins to

a level where the body is not able to neutralize. Thus the quantity of plaque is directly proportional to the disease status[15]. This theory was, however, challenged as some individuals showed signs of advanced disease progression with minimal plaque accumulation while others showed extensive plaque buildup with minimal progression.

This led to the emergence of another theory termed *specific plaque hypothesis*. This theory stated that not all plaque has the same pathologic effect on the host and that some bacteria are more pathogenic than others. This was shown with association studies through DNA culture. Bacteria associated with more severe forms of disease progression were termed periodontal pathogens and assigned in different complexes [16].

Plaque induced gingivitis can also be exacerbated by systemic and local factors that must be controlled for the resolution of inflammation.[17]

Among the common clinical signs of gingivitis is the presence of erythema in the marginal and the attached gingiva as well as the presence of edema (increase in size), alteration of shape, and bleeding upon probing with no apical migration of the attachment apparatus, which is the epithelial attachment and connective tissue attachment maintaining the gingival attachment at or coronal to the cementoenamel junction[7, 17]. Histologically gingivitis presents with a high infiltration of lymphocytes at the site of the connective tissue with increased leukocytes at the junctional epithelium and vasculitis accompanied with loss of collagen fibers[18].

Gingivitis can also be a precursor to periodontitis, which involves the apical migration of the

supra crestal attachment apparatus, usually tooth-supporting bone loss, and potential tooth loss. Gingivitis was found to be an important risk factor for periodontitis [19]. Thus multiple studies have proposed that the early diagnosis and treatment of gingivitis and prevention can be key to establishing clinical periodontal health. Numerous longitudinal studies showed that around 10% of the population have a strong susceptibility to advanced periodontal disease progression [Grade C] [20]. Genes were studied as genetics is considered to have a possible role in the susceptibility to periodontal diseases. Gene polymorphisms were found between different ethnic groups and were studied in multiple association studies. For example IL-1 gene polymorphism was found to have an effect on the susceptibility of localized aggressive periodontitis in the Asian population[21]. Many other genes are also being studied for their role in susceptibility to periodontal diseases. On an epigenetic level, recent findings have shown that bacteria have the potential to cause alterations in cellular DNA methylation status. This along with effects of environment, aging, and stress can lead to epigenetic modifications that modify gene expression and disease expression[22]. While the direct effect of bacteria on the host immune inflammatory response is studied extensively, its effect on various epigenetic pathways involved in the modulation of inflammatory and anti-inflammatory genes is still not clear.

With several definitions, studies have shown a great amount of heterogeneity with what should be termed a, “gingivitis case”. With various definitions, the prevalence of gingivitis has reached up to 90%[23]. Not only is the severity of the disease a factor in this heterogeneity but also the extent of the disease whether involving one site or a certain percentage of sites. Many indices have been established to evaluate the severity of gingivitis utilizing the clinical

presentation as well as probing to evaluate the signs of inflammation.

Bleeding on Probing:

The periodontal probe has long been used as a key diagnostic tool in detecting gingival and periodontal conditions. It is primarily used to measure the sulcus depth. It is known that the depth of the penetration of the probe will vary depending on probing pressure, diameter of the probe and the health of the gingiva[24]. In clinical health when the probe force is standardized to 0.25N and probe diameter to 0.5mm the probe tip was shown to stop at the apical extent of junctional epithelium coronal to the insertion of intact connective tissue fibers[25]. Probing is not only used to record depth but also to record tissue response after probe penetration. As there are no blood vessels in epithelium, the probe does not cause bleeding in an intact, healthy gingival sulcus.

Based on the histological findings of the disease, a bleeding score is of great importance in detecting inflammation.[26, 27] The Papillary Bleeding Index (PBI) was evaluated histologically, and it was shown that as bleeding increased in intensity, the size of the leukocyte-infiltrated connective tissue segment, and the density of the inflammatory infiltrate increased as well[28]. The sites which bled after probing had a significantly greater areas of inflamed connective tissue compared with the sites that had no evidence of bleeding[29]. Gingival sites with visible signs of inflammation were not shown to have significantly increased blood flow and the blood velocity in capillaries of inflamed gingiva did not increase[30]. This increased bleeding tendency inflamed sites is due to ulcerations in the epithelium, which result in

alterations in the blood vessel walls allowing the capillaries to be more permeable and thus likely to bleed upon probing. With the idea that BoP is an indicator for gingival inflammation, BoP was tested for its reliability as a diagnostic tool. BoP as a diagnostic method for predicting periodontal stability was shown to have low sensitivity; however, it has a very high specificity, which indicates that the absence of BoP can be a reliable indicator for periodontal stability [26, 27, 29]. However, this may not be the case for smokers as studies have showed that smoking can impair the vascularization of soft tissues and neutrophil function with depressing lymphocyte count and resulting in impaired wound healing [31-33].

In order to decrease heterogeneity with defining clinical gingivitis among dental practitioners, a good gingival index should be as free as possible of subjective interpretation[27]. A visual examination has a degree of subjectivity, because it quantitates the degree of inflammation visually. To minimize possible subjectivity and bias, gingival indices were developed that use a binary presence or absence of bleeding as a sign of gingival inflammation [34].Thus, BoP can be used as an objective tool for detecting gingival inflammation[26].

Reducing dental plaque:

With dental plaque being the major etiological factor in the disease process, methods of reducing dental plaque accumulation both professionally and at home were researched to achieve a level of clinical health.

Tooth brushing plays a significant role in reducing plaque accumulation on teeth and decreasing gingival inflammation[35-40] .However, while tooth brushing is able to remove

cervical and occlusal plaque, interproximal sites (sites between the teeth) especially subgingivally are not efficiently accessed or cleaned by the toothbrush bristles in patients with interproximal contacts[41].

Many tools and devices are marketed as adjuncts to help to remove interdental plaque. The most common of these are: dental floss/tape, inter dental brushes, toothpicks, rubber tips, and oral irrigators. Studies have shown that interdental brushes are the most effective tool in removing interdental plaque[42]. A major drawback is that they do not fit in all embrasures (spaces between teeth) especially those of healthy patients when there is no interdental gingival recession. Other advantages of dental floss over alternate interdental plaque removal aids is the depth in which the floss can penetrate subgingival to dislodge dental plaque. Dental floss has a much smaller diameter which allows it with a proper technique to penetrate further in the gingival sulcus. Additionally the force applied by a floss, if utilizing a correct flossing technique, is directed perpendicular to tooth surface[43]. This gives it better capability of dislodging dental plaque as compared with an interdental brush. The bristles of an interproximal brush are used with a faciolingual (scrub-like) brushing stroke and are generally parallel to the tooth, which might not provide adequate force to dislodge subgingival plaque. Additionally the length of the bristles of interdental brushes might not access the tooth in an apical dimension, as most healthy interproximal crevices are from 2-3mm.

The debate over the dental floss:

The effect of flossing on reducing gingival inflammation has been debated in the literature with multiple studies showing positive outcomes for the addition of flossing to tooth brushing and others showing no additional benefit[44].

A more recent meta review by Salzer et al in 2015 concluded that the beneficial effect of flossing is not supported by scientific evidence[42]. This was based on the findings of two articles.

The first is a 2008 systematic review looking into 11 publications comparing the addition of flossing to tooth brushing on plaque and gingival inflammation. The authors concluded that ‘‘routine instruction to use floss is not supported by scientific evidence” [44]. In those 11 publications it was noted that there was no specific instruction given for flossing and two trials used followed instructions by the ADA. Most studies also used the Löe and Silness gingival index [45](7 studies) and four studies used BoP scores. In most studies gingival inflammation was diagnosed around the entire circumference of the tooth as most of the studies had tooth brushing only as part of the study. Only one study showed significant gingival index results however, with the four studies that looked at BoP, two studies showed significant improvement with flossing[46, 47]. The two other studies that did not show a statistical significance in BoP reduction with the addition of using dental floss, still showed an 11% and a 13% increase in BoP reduction compared with tooth brushing alone[48, 49]. While statistically the meta-analysis did not find additional benefit to flossing on the improvement of gingival inflammation as evaluated by the Gingival Index with a p value of 0.06, clinically there seemed to be a reduction in BoP scores in all four articles that evaluated BoP scores with the use of floss over brushing without flossing.

A 2011 Cochrane review that looked at 12 clinical trials. A meta-analysis was conducted on gingivitis index scores and not bleeding scores as not all trials reported bleeding index. The meta-analysis concluded that there is some evidence that flossing plus tooth brushing can decrease signs of gingival inflammation compared with tooth brushing alone at 1,3,and 6 month

time intervals; however there was much weaker evidence on the ability of flossing to reduce plaque[50].

A survey study conducted in hospital and private practice settings found that the prevalence of flossing can only reach as high as 20% and of those only 40% presented with acceptable flossing techniques [51]. Accepted flossing techniques were defined according to a questionnaire given to the participants in which they responded to the number of times going up and down between the teeth and if they pull the floss around the sides of the tooth. They also found using the questionnaire that 39.5% of participants who reported yes to flossing reported no to flossing around teeth[51]. Another study using an in-home dental examination of over 300 participants showed that a third of the population was flossing. An important finding was that only 22% of participants who reported flossing, satisfied the criteria for an acceptable flossing technique [52]. Clinicians still seem to believe in the importance of flossing and still recommend it to patients on a daily basis even though it is weakly backed by scientific evidence[42].

A reasoning for this lack of consistency between research and clinical effectiveness of daily flossing can be attributed to the lack of specific flossing instructions provided to the patients and the lack of compliance by the patients as well as lack of motivation. Research has found that people do not usually floss in a correct manner. The heterogeneity in the results of the effectiveness of flossing in reducing plaque can be attributed to the lack of explanation in the studies as to the technique of flossing that was being taught, was there an attempt to teach a specific instruction, and were the study participants in compliance with the specific flossing technique instructed?

To best of our knowledge there is no specific flossing technique proven to be the most effective and easily performed by patients. The ADA recommends to floss all the teeth by providing this specific set of instructions which is: To “use about 18 inches of floss around one of your middle fingers, with the rest wound around the opposite middle finger”. To “hold the floss tightly between the thumbs and forefingers and gently insert it between the teeth”. To “curve the floss into a “C” shape against the side of the tooth”. To “Rub the floss gently up and down, keeping it pressed against the tooth”.

Thus, for this study, one aim was to investigate if instructed dental flossing with a specific technique [53] is effective in reducing inflammation as evidenced by BoP. The technique employed was similar to the technique recommended by ADA with the addition that the floss should be moved in a short buccal-lingual motion as well as apical-occlusal motion as if “one were drying their back with a towel”[53].

Flossing is regularly performed by dental hygienists for their patients as part of a dental prophylaxis[54]. However, it is not known whether professional flossing is superior to the patient’s self-flossing in removing plaque or reducing gingival inflammation. In the United States, data from NHANES 2011-2012, 2013-14 showed the prevalence of flossing among adults to be at 31.6%[55].

Not only is the specific technique of flossing very important but also how to instruct the patient is critical. One study looked at four methods for oral hygiene instructions. They tested

oral individualized instruction, oral standardized instruction, and written instructions. They found an advantage of oral individualized instructions compared to other methods which was shown by significantly less bleeding after the intervention[56].

Aim and Hypothesis:

The main aim of this study was to test the effectiveness of using dental floss daily with specific instructions and with a fixed frequency interval. This is compared with subjects flossing at their usual interval without flossing instructions to determine the degree of gingival inflammation as evidenced by BoP.

A second aim was to compare the completeness of plaque removal of flossing by a dental professional with the completeness of a subject's self-flossing following instruction with the specific technique as evidenced by using disclosing pills at various time periods (initial and after 2 months).

The hypotheses were: 1) Instructed daily flossing for subjects who exhibit gingivitis reduces bleeding on probing (BoP) of interdental sites more than for subjects who are uninstructed and floss at their usual intervals. 2) Professional flossing reduces interdental plaque more effectively than allowing the subjects to floss for themselves. 3) Instructed subjects can remove more interproximal plaque after practicing the techniques daily for 2 months than they could when initially instructed. 4) The instructed subjects and the clinician will be equally complete with plaque removal at the fourth clinical evaluation.

II- Materials and Methods

Thirty-six participants were recruited based on a power calculation using the statistical software nQuery (Version 7.0) to estimate the statistical power of the study. The results of Walsh and Heckman [47] were employed to obtain the anticipated effect size. Based on these results, a final sample size of n = 14 per group yielded a power of 99% at the $\alpha = 5\%$ significance level. To account for approximately 20% attrition, the sample size was set at n = 18 per group (for a total of 36 subjects treated).

The study design used was a randomized controlled clinical trial. Specifically, it was two-group parallel, 8-week home-use study, where 36 subjects were assigned to one of two groups; Group A (Test) or Group B (Control).

The groups were computer randomized using the "sample" function of the statistical software package R (Version 3.3.1). Group A was instructed with a specific technique of flossing and asked to floss once daily. Group B was asked to continue with their regular dental hygiene techniques. The primary examiners were blinded during each examination visit. A trained instructor gave the flossing instructions to Group A at the initial visit and subsequent visits. Thereafter a primary blinded examiner measured bleeding scores for all subsequent visits. Three blinded examiners were calibrated on probing.

Calibration was done as follows:

1) The PI initially chose one quadrant to be examined for probing depths

2) The PI proceeded to examine the one quadrant to be used for probing depth measurements twice and an assistant recorded the depths. Both sets of chartings had to be within 1mm of each other. These numbers were used as the standard.
3) The PI recorded, and the 3 potential examiners examined the one quadrant of the mouth for probing depths that the PI measured.
4) All 3 potential examiners examined the same two quadrants for probing depths
5) The recorded results were compared and the examiners needed to be within 95% agreement. That is, 95% of probing measurements at a particular visit had to be within 1mm apart in probing depth between all calibrated examiners and the PI
6) Should an examiner not achieve 95% agreement with the PI the process was repeated until the 95% level was reached.

Subjects' inclusion criteria were:

- At least 18 years of age
- Subjects must have showed > 20% interproximal sites (mesial facial and mesial lingual and distal facial and distal lingual) with BoP. This includes mesial and distal sites next to edentulous areas.
- Subjects must have had > 24 natural teeth including teeth with crowns with margins confluent with the natural tooth as long as they do not violate the biologic width.

- Third molars were excluded from study, unless the third molars are in the anatomical position of second molars. However, subjects with third molars present will be accepted into the study unless there are other factors that excluded them.
- Subjects must have had a professional dental prophylaxis within four months of beginning the study
- Subjects must be willing to floss daily and to be taught and use a specific technique of flossing.

Exclusion criteria:

- Subjects who used tobacco products.
- Subjects with fixed or removable orthodontic appliances

  Subjects with splinted fixed prostheses
- Subjects who are incapable of flossing their teeth
- Subjects with defective interproximal restorations or interproximal caries that are clinically evident and impacting gingival health.
- Subjects where malposed teeth preclude the use of floss
- Subjects with probing depth greater than or equal to 5mm.
- Subjects who are pregnant (self-reported) due to the greater bleeding tendency due to the hormonal changes.
- Subjects with a systemic disease that affects the gingiva or are taking medications of which affect the gingiva.
- Non-English speaking subjects, as study assessments and instructions will be in English only.

Subject were withdrawn if they did not comply with the study procedures or for any reason chose to stop participating in the study.

Study procedures:

Subjects were asked to read the informed consent form (ICF). Subjects were given ample time to have any questions answered. If a subject decided to participate, he/she was instructed to sign the ICF. A copy of the ICF was given to the subject. He/she was asked to complete demographic information and a medical, dental, and social history form. (Appendix I)

The medical and dental history was verbally reviewed, and they were asked verbally about their oral hygiene habits. An oral examination, including an evaluation of the oral cavity, an assessment of BoP and probing depth was done along as per standard of care. A periodontal probe (UNC 1-15 mm) was used to measure gingival pocket depths. All examination findings were recorded. Probing depths (PD) were recorded on six sites per tooth (mesial, mid cervical and distal on both facial and lingual surfaces of each tooth) excluding third molars.

Modification of the gingival bleeding index[57], indicating whether there was or was not bleeding in the interdental gingival crevices, was utilized to obtain a BoP score. The examiner gently swiped the probe at the most apical portion of the gingival crevice from line angle to line angle interproximally and after 10 seconds assessed if there was bleeding or no bleeding on the mesial (facial and lingual) and the distal (facial and lingual). Once a subject fulfilled all criteria to be in the study, the evaluator used the randomization scheme and thus randomly assigned the subject to one of two groups (Group A test, Group B control).

Group A- Visit 1:

At Visit 1 participants in group A had their teeth stained with a plaque disclosing dye. The subjects chewed a Sunstar Butler GUM™ disclosing tablet, and once dissolved in the saliva they swished it in their mouth for 30 seconds. Then they expectorated and rinsed their mouth with water. A plaque index (Modified O'Leary Plaque Score Index Chart)[58] was recorded in which the all teeth surface are stained using a plaque disclosing dye. Plaque was marked as whether present or absent interproximally only for each tooth (buccal and lingual surfaces).

Subjects were provided with dental floss (GUM Butlerweave ™) and asked to floss their teeth as they normally do. The evaluator observed the subject's flossing techniques, which was recorded. Participants were defined as having adequate technique if they were fully adapting the floss to the proximal sides including the line angles of the teeth using their thumb and index finger while moving the floss in a vertical and horizontal motion to dislodge interdental plaque. Participants in both groups were asked to demonstrate their technique at each study visit and were classified in two categories: successful (able to follow the exact study technique) and unsuccessful (not able to follow the exact study technique). Participants in Group A received professional instructions on the technique of flossing according to written and visual instruction as described above under "adequate technique" [53]. At the outset, the clinician demonstrated the flossing on a dental model for the subject. The subject then demonstrated what the clinician showed on the dental model. Then the clinician flossed the subject's teeth on one side (half) of the mouth maxillary and mandibular. A computer-generated randomization list was followed using the "sample" function of the statistical software package R (Version 3.3.1) to decide which side of the mouth would receive professional flossing. The study subject was instructed to

observe the professional flossing in a patient mirror. Following this, the participant flossed the other side (half) of the mouth to demonstrate their understanding of the technique. The teeth were re-stained as above and the Modified O'Leary Plaque Score was determined for all teeth on both sides of the mouth. The subjects were offered a Sunstar Butler manual toothbrush to remove the stained plaque if they wished to remove it at that time.

Subjects in Group A were asked to floss once a day using the specific technique demonstrated utilizing the same floss (GUM Butlerweave ™). They were asked to maintain a flossing and brushing diary, which was given to them at end of the visit. Group A was asked to refrain from any other interproximal hygiene techniques or devices other than the specific dental floss provided. The subjects were asked to floss one time per day, and all dental hygiene techniques were recorded in their diary. For tooth brushing, the subjects were asked to continue using their tooth brushing techniques as they had been doing prior to entering into this study. Oral hygiene instructions were provided verbally and on paper as seen in Appendix B

Group B - Visit 1:

As for the control group (Group B), dental plaque was stained with a plaque disclosing dye. The same scoring technique as described for Group A was done and recorded (O'Leary Plaque Score). Subjects were provided with dental floss and asked to floss their teeth as normal. The evaluator observed their flossing technique, which was recorded in a similar manner as with Group A as mentioned above. The subjects were offered a manual toothbrush to remove the stained plaque if they wished to remove it at that time. The subjects were asked to continue their

habits or techniques of their dental hygiene, as they reported and demonstrated at the initial examination. Subjects were not provided with any instructions for flossing.

Both groups were asked not to use other interproximal cleaning devices during the study period. Oral hygiene instructions were provided verbally and on paper as seen in Appendix B.

Groups (A, B) – Visit 2:

Visit 2 was conducted after two weeks after visit 1. The medical history was reviewed and any changes were noted. Subjects were asked verbally about their oral hygiene habits. The eligibility and subject withdrawal criteria were reviewed to ensure the subject still qualified for the study. The flossing and brushing diaries for the subjects in Group A were evaluated and collected to ensure that the subject was compliant with the study protocol. The blinded examiner recorded BoP for both Groups A and B and the plaque score (Modified O'Leary Plaque Score). Flossing techniques were observed for Group A only, and flossing technique instructions were corrected as needed by a non-blinded study team member.

Groups (A, B) – Visit 3:

Visit 3 was conducted after two weeks after visit 2. The medical history was reviewed and any changes were noted. Subjects were asked verbally about their oral hygiene habits. The eligibility and subject withdrawal criteria were reviewed to ensure the subject still qualified for the study. The subjects' diaries in Group A were checked and collected to ensure they were compliant with the study protocol. New diaries were given to the subjects in Group A. The blinded examiner

recorded BoP for both Groups A and B and the plaque score (Modified O'Leary Plaque Score). Flossing and brushing techniques were observed for Group A only, and flossing technique instructions were corrected as needed by a calibrated non-blinded study team member.

Groups (A, B) – Visit 4:

Visit 4 was conducted after four weeks after visit 3. The medical history was reviewed and any changes were noted. Subjects were asked verbally about their oral hygiene habits. The eligibility and subject withdrawal criteria were reviewed to ensure the subject still qualified for the study. The subjects' diaries in Group A were checked and collected to ensure they were compliant with the study protocol. The blinded and calibrated examiner recorded BoP for both Groups A and B.

Group A:

Plaque was stained with a plaque disclosing dye as done at the previous visits. Plaque index (Modified O'Leary Plaque Score) was recorded interproximally for each tooth. The clinician flossed the subject's teeth on one side (half) of the mouth maxillary and mandibular. Following this, the patient flossed the other side (half) of the mouth to show their understanding of the technique. Modified O'Leary Plaque Score was scored for all teeth on both sides of the mouth. The subjects were offered a manual toothbrush to remove the stained plaque.

Group B

Plaque was stained with a plaque disclosing dye. Plaque index (Modified O'Leary Plaque Score) was recorded interproximally for each tooth. Professional instructions were given by an investigator on the technique of flossing according to written and visual instructions. At the outset, the clinician demonstrated the flossing on a dental model for the subject. The subject demonstrated

what the clinician showed on the dental model. Then the clinician flossed the subject's teeth on one side (half) of the mouth maxillary and mandibular. The study subject was instructed to observe the professional flossing in a patient mirror. Following this, the patient flossed the other side (half) of the mouth to show their understanding of the technique. Plaque score was recorded after flossing using Modified O'Leary Plaque Score. The subjects were offered a manual toothbrush to remove the stained plaque.

Statistical Analysis:

Descriptive statistics were calculated (means, medians, standard deviations, and inter-quartile ranges for continuous variables; counts and percentages for categorical variables). Independent-samples t-test was used to compare baseline age between both groups. Chi-square test was used to compare gender differences between both groups.

Flossing analysis:

Cochran's Q test was used to compare group A and group B's ability to learn the technique and successfully perform it over all time points. Further analysis was done using McNemar's test to compare any difference in the ability to learn the technique between different time points. Bonferroni correction was used to detect statistical significance.

BoP analysis:

Baseline percentages of BoP were compared via the Mann-Whitney U test. Independent-samples t-tests were used to compare the two groups in terms of change in the percentage of sites with BoP. Analysis of covariance (ANCOVA) was also employed to adjust for age, gender, and baseline percentage of sites with BoP. Friedman's was used for intra-group comparisons of percentage of BoP over time. Wilcoxon signed-rank test was used to compare any intra group differences in terms of change of BoP at different time points. Bonferroni correction was used to detect statistical significance.

Plaque analysis:

The paired t-test was used to compare the clinician and subject in terms of change in plaque score at each time point, as well as to compare change at first examination with change at last examination (for clinicians and subjects separately). For Group A, the comparison of the first visit and the final visit in terms of the difference in plaque removal between the clinician

and the subject was conducted. When data set was not normally distributed a Wilcoxon signed-rank test was used.

The significance level was set at $\alpha = 5\%$. The analysis was conducted using SPSS Version 24.

III- Results

Thirty-six participants were recruited for the study. 2 participants failed to follow-up for all 4 visits of the study and were excluded. Thirty-four participants completed all four visits, and thus the statistics were compiled based on 34 participants, 17 in each group. Initial demographics are shown in Table 1.

Flossing Technique

At the outset of the study, all participants were asked to demonstrate the flossing technique they usually used to remove interproximal plaque. The participants used mixed variations of technique starting from an apical coronal motion breaking the contact area with floss to remove food particles out, to multiple vertical motions with no adapting, to semi adapting the floss in a vertical motion only. The illustrated graphs show the participants of both groups' progress in learning the technique. (Fig 1,2). All subjects in Group A presented with different flossing techniques than the technique being instructed. On the second visit, 10 out of 17 (58.8%) were able to demonstrate flossing in the manner they were instructed on the first visit. On the third visit 13 out of 17 (76.5%) were able to demonstrate the instructed flossing technique after the instructions were reinforced on the second visit. On the final visit, 15 out of 17 (88.2%) were able to demonstrate the instructed flossing technique adequately; only 2 participants were not able to fully adapt the floss around the posterior area of the mouth following the 2 months of instructional flossing. Based on Cochran's Q test, the difference between time points was statistically significant ($P < 0.001$). In post-hoc testing via McNemar's test with the Bonferroni correction, significant differences were found between baseline and visit

2, baseline and visit 3, and baseline and visit 4 (all $P < 0.001$); other differences were not significant (Table 2).

All subjects in Group B presented with different flossing techniques than the technique being instructed. On the second visit and third visit no flossing instructions were taught. On the final visit, participants were taught the flossing technique which was taught to Group A and asked to demonstrate it after one round of practice. 8 out of 17 (47.1%) were able to demonstrate the instructed flossing technique adequately. Per Cochran's Q test, the difference between time points was statistically significant ($P < 0.001$). In post-hoc tests using McNemar's test with the Bonferroni correction, differences between the final visit and all other time points were statistically significant ($P = 0.007$); other differences were not significant (Table 3).

Bleeding Scores

All subjects were tested at baseline for interproximal bleeding scores using the Gingival Bleeding Index, indicating whether there was or was not bleeding in the interdental gingival crevices. Baseline results are shown in Table 4. No statistically significant difference in baseline BoP was observed between the groups ($P = 0.208$).

Subjects in Group A had a mean interproximal BoP of 25.9% at baseline. On the second visit the mean BoP was 8.2%; the mean further decreased to 7.1% on Visit 3 and then slightly increased to 7.9% at Visit 4 (Fig 4). Based on Friedman's test, the difference between visits was statistically significant for this group ($P < 0.001$) (Table 5). Post-hoc Wilcoxon signed-rank tests with the Bonferroni correction were done to evaluate statistical significance between the different time points. Statistical significance was found between baseline and Visit 2 ($P = 0.002$), baseline and Visit 3 ($P < 0.001$), and baseline and Visit 4 ($P < 0.001$) (Table 6).

Subjects in Group B had a mean interproximal BoP of 24.45% at baseline. On the second visit the mean BoP was 15.6%; it further decreased to 12.3% on Visit 3 and then increased to 18.0% at Visit 4 (Fig 5). Friedman's test determined that the difference between visits was statistically significant (Table 7). Post-hoc Wilcoxon signed-rank tests with the Bonferroni correction were done to evaluate statistical significance between the different time points. Statistical significance was found between baseline at Visit 2 ($P = 0.003$) and between baseline and Visit 3 ($P < 0.001$); no other test was significant when applying the Bonferroni correction, which resulted in a significance level of $\alpha = 0.0083$ (Table 8).

Subjects in both groups were further compared in terms of reduction in BoP from baseline to the final visit. Group A showed a mean reduction of 18.1% of sites while Group B showed a mean reduction of 6.4% of sites. These numbers were statistically analyzed using the independent-samples t-test and the difference between the two groups was found to be significant ($P = 0.034$). Further analysis was done to adjust for baseline BoP, age and gender using ANCOVA and the difference was again found to be statistically significant ($P < 0.001$) (Table 9).

Plaque Scores:

Group A

Subjects in Group A were stained for plaque at baseline using the Modified O'Leary Plaque Score Index Chart. After the flossing technique was taught the instructor demonstrated flossing a randomly assigned half the mouth and the participant flossed the other half. Based on the paired t-test, no statistically significant difference was shown between baseline plaque scores on the subjects' vs. the instructor's sides ($P = 0.948$) (Table 10).

Statistical analysis was done to compare the instructor's side and the subjects' side in terms of reduction in plaque scores after flossing. On Visit 1, the instructor was able to make 47.6% of interproximal sites with plaque, plaque free on the half of the mouth that they flossed. The subject was able to make 38.3% of interproximal sites with plaque, plaque free on the other half of the mouth that they flossed. A paired t-test was used and the result was statistically significant (P = 0.024) (Table 11).

Group A was stained again for plaque at the final visit (Visit 4). Based on the paired t-test, no statistically significant difference was shown between baseline plaque scores on the subject and instructor sides (P = 0.323) (Table 12). The same study design was repeated whereby the instructor demonstrated flossing a randomly assigned half of the mouth and the participant flossed the other half. On Visit 4, the instructor was able to make 42.3% of interproximal sites with plaque, plaque free on the half of the mouth that they flossed. The subject was able to make 39.4% of interproximal sites with plaque, plaque free on the other half of the mouth that they flossed. A Wilcoxon signed-rank test was used and the result was not statistically significant (P = 0.276) (Table 13).

Further analysis was conducted to compare the completeness of plaque removal by the subjects in Group A in their first and last visit. On their first visit a mean plaque reduction of 38.2% was achieved while on their last visit it was 39.4%. Using a Wilcoxon signed-rank test the results were not statistically significant, (P = 0.722) (Table 14).

A comparison of Visit 1 vs Visit 4 in terms of (instructor % plaque removal - subjects' % plaque removal) was done to analyze the proximity of the subjects' efficacy of flossing compared to the examiner at baseline and time point 4. The instructor outperformed the subject by 9.3% at

baseline while this difference dropped to 2.8% at Visit 4. Using a paired t-test, the results were not statistically significant (P = 0.147) (Table 15).

Group B

Subjects in Group B were stained for plaque at Visit 4 using the Modified O'Leary Plaque Score Index Chart. After the flossing technique was taught the instructor demonstrated flossing a randomly assigned half the mouth and the participant flossed the other half. No statistically significant difference was shown between baseline plaque scores on the subjects' and instructor's sides (P = 0.932) (Table 16).

A statistical analysis was done to compare the instructor's side and the subject's side in terms of reduction in plaque scores after flossing. On Visit 4, the instructor was able to remove a mean of 47.1% of interproximal plaque sites on half the mouth making them plaque free. The subject was able to remove a mean of 38.0% of interproximal plaque sites making them plaque free on the other half. A paired t-test was used and the result was statistically significant (P = 0.005) (Table 17). This was similar to Group A at their first visit.

IV- **Discussion**

Flossing Technique

The present clinical trial's main aim was to evaluate whether daily flossing with a specific instructed technique and a fixed frequency interval is more effective in reducing gingival inflammation as evidenced by bleeding on probing (BoP) compared with non-instructed flossing. A survey study conducted in a hospital and private practice setting found that the prevalence of flossing can only reach as high as 20% and of those only 40% presented with acceptable flossing techniques [51]. Multiple studies have shown flossing not to have a statistical significance in terms of reduction of gingival inflammation. A possible explanation is lack of quality instruction and application of instruction by participants.

In this study, all participants were asked to demonstrate the flossing technique they used to remove interproximal plaque before starting the study. None of the 34 participants used the same flossing technique as was recommended by the study. Fifteen out of 34 (44.1%) used a vertical motion to apply floss without any attempt to adapt the floss to the proximal surfaces of the teeth. The rest of the participants attempted to adapt the floss with varying levels of completeness in efficient plaque removal. This is in accordance with Segelnick et al [51] whose survey showed that only 40% of participants attempted adapting the floss around the proximal surfaces of teeth. Subjects in Group A who were taught the technique described for this study and had re-reinforced at every time point showed an improved level of understanding of the technique, and by the fourth visit, 88.2% of subjects were comfortable with the flossing technique; only 2 participants failed to learn to apply the technique adequately in 2 months. This significant improvement was also shown in a study by Radentez et al. The study found that subjects could

floss only 7.5% of selected dentition and after receiving video-taped instructions along with chair side instruction the number of teeth flossed properly increases to 94%[59]. The participants in Group B in this study showed no signs of learning the technique on their own throughout the first three study visits, and after having the technique explained chairside at the final visit, 47.1% were able to demonstrate the instructed flossing technique adequately across the entire dentition. This could be explained as the majority of participants struggled adapting the floss to the interproximal surfaces of the posterior teeth and thus did not qualify in a category of fully practicing the technique even though their technique improved significantly.

Bleeding on Probing

Subjects in Group A started at baseline with a mean interproximal BoP of 25.9%. On the second visit the mean BoP was 8.2% and further decreased to 7.1% on visit 3 and then slightly increased to 7.9% at visit 4. This was in association with the subjects' ability to learn the technique and perform it adequately, as by the second visit 59% of subjects were performing the technique adequately. On average subjects had an 18.1% reduction of the total (bleeding and non-bleeding) number of sites that bled upon probing, which can also be interpreted that of the 69.55% sites that initially were bleeding, did not bleed following 8 weeks of instructed daily dental flossing. This is accordance with study by Graves et al that showed similar 67-69% BoP reduction[60]. In a 2008 systematic review [44] that studied the effect of flossing in managing gingivitis, four studies calculated BoP with and without flossing. Two studies showed a significant improvement with flossing by showing a reduction of 12% of sites and reduction of 0.42 score with the PBI index [46, 47] . The two other studies that did not show a statistical significance with BoP reduction with the addition of floss still showed an 11% and a 13% increase in BoP reduction compared with

tooth brushing alone[48, 49]. The results of the previously mentioned studies were similar to the participants in Group B who used dental floss without receiving instructions. The participants in Group B showed a mean 6.4% reduction in the total number of sites that bled upon probing, which can also be interpreted that 26.3% of sites that initially were bleeding did not after 8 weeks of non-instructed dental flossing.

The aforementioned results show that instructed dental flossing is effective in decreasing the percentage of bleeding interproximal sites. While non-instructed flossing was also effective, the magnitude of the effect was significantly increased when performed daily with a particular technique that emphasizes adapting the floss to the proximal surfaces and line angles of the teeth. This was also in accordance with a recent meta-analysis [61] that showed a small yet positive effect of flossing in reducing BoP. However, concluded that unsupervised flossing can be of minimal benefit.

According to the data, patients who received flossing instructions exhibit less bleeding on probing, as opposed to those patients who did not receive flossing instructions. This can explain why previous studies that tested efficacy of flossing without providing clear instructions were not able to find a clinical and statistical significance to the addition of flossing in the daily home care regimen.

Plaque

The effect of flossing on reducing interdental plaque has been debated in the literature [44, 50]. This may be due to inadequate techniques patients use to remove plaque with a dental floss. Flossing can be regarded as a skill, and a lack of this skill often can be attributed to a lack of motivation. The absence of flossing may also be due to a lack of manual dexterity [62]. Parents

flossing their children's teeth reduces interdental plaque more effectively than allowing children to floss for themselves[63]. Thus this study used flossing by a dental professional as a gold standard and compared it with subjects after they received flossing instructions using a split mouth design. Subjects in Group A at Visit 1 after receiving flossing instructors, were able to remove a mean value of 38.3% of sites with interproximal plaque making them plaque free while on the other half of the mouth, the instructor was able to remove a mean value of 47.5% of sites with interproximal plaque. This suggests that even after instructions an experienced instructor is better at utilizing the dental floss in removing interdental plaque than the instructed subject. A limitation was that the plaque score used only looked at presence or absence of interdental plaque and did not measure the reduction in quantity of plaque per site. In cases where clinicians and subjects were not able to transform sites with stainable plaque to plaque free sites, most sites still showed a reduction in amount of plaque even if not rendering them all plaque free.

Subjects in Group A were also tested using the same split mouth design at visit 4. The instructor was able to remove a mean of 42.2% of interproximal plaque sites on half the mouth making them plaque free. The subject was able to remove a mean of 39.4% of interproximal plaque sites. This, however, failed to show that participants were able to improve in their plaque removal efficacy between the day they learned the technique following 2 months of their practicing. This might be due to not having a large enough sample size since the criterion being tested was a subject's ability to remove plaque after being instructed on the same day and after two months. Another possible reason is that the plaque score used only considered presence or absence of plaque and did not consider the quantity of plaque removal. Areas with root concavities may present limitations with regards to complete plaque removal with dental floss since the floss would

not completely adapt to remove the entire amount of plaque. A limitation to the study was that subjects were never tested regarding their ability to remove plaque before they received instructions by a professional. Such testing would have shown the within-subject difference between instructed and non-instructed flossing in terms of the amount of plaque removal. As for the difference between professional flossing by an experienced instructor and by a subject, at visit 1 there was a statistically significant difference while no statistical significance was observed at visit 4 in the amount of sites plaque free after flossing.

Subjects in Group B at Visit 4 received flossing instructions and followed the same experimental design as subjects in Group A at visit 1. After receiving flossing instructions, the subject was able to remove a mean of 38.0% of interproximal plaque sites making them plaque free while on the other half the instructor was able to remove a mean of 47.1% of interproximal plaque sites. This showed the same findings with Group A subjects at their first visit. Even after instructions an experienced instructor was still better at utilizing the dental floss in removing interdental plaque.

Previous studies have showed that flossing might have limited benefit in terms of improving gingival and bleeding scores [44, 50]. These findings were the basis for the United States Department of Health and Human Services to remove the recommendation for daily flossing from the Federal Dietary Guidelines released in 2015. The present study was able to find an additional benefit to instructed dental flossing opposed to just flossing without any given instructions. This can help explain why multiple previous studies failed to show significant improvements. Previous studies lacked a scientifically proven specific technique on how to floss.

Although the removal of plaque is important, the quantity of plaque removal was not studied in this investigation, the reduction of BoP is most important. The toxins from bacterial plaque are etiologic factors in the presence of gingival inflammation. The length of time and concentration of these contents in contact with the gingival cells is critical in the breakdown of epithelial cells and the initiation of gingival inflammation. Thus the presence of stainable plaque is temporal, and BoP is a more reliable marker for inflammation than the presence of plaque. Based on our findings the habit of flossing is more like practicing a sport. It will require multiple coaching visits with the right technique and constant reinforcement by dentists and dental hygienists. By doing so, patients can truly benefit from the mechanical ability of the floss to disrupt the oral biofilm and achieve the end result of clinical gingival health.

Conclusion

Within the limitations of the study, daily instructed flossing with the floss adapted to the proximal surfaces of the teeth using a simultaneous horizontal and vertical motion is more effective in the reduction of interproximal BoP compared with individuals who were not instructed to floss daily and without flossing instruction. Many subjects who were taught the flossing technique and did not accurately perform that technique upon the first trial, required a learning phase with repeated instruction. This study illustrates the importance of technique instruction at the initial visit and reinforcement over a longer period of time. Regarding plaque removal, the current study failed to show a clinically significant difference between an instructor performing the flossing technique and subjects performing the flossing technique. Both subjects and instructor were able to remove plaque with the flossing technique described in up to 50% of sites. Future multi-centers studies with an increased sample size and follow- up time are recommended to confirm the current findings.

Appendix A Figures:

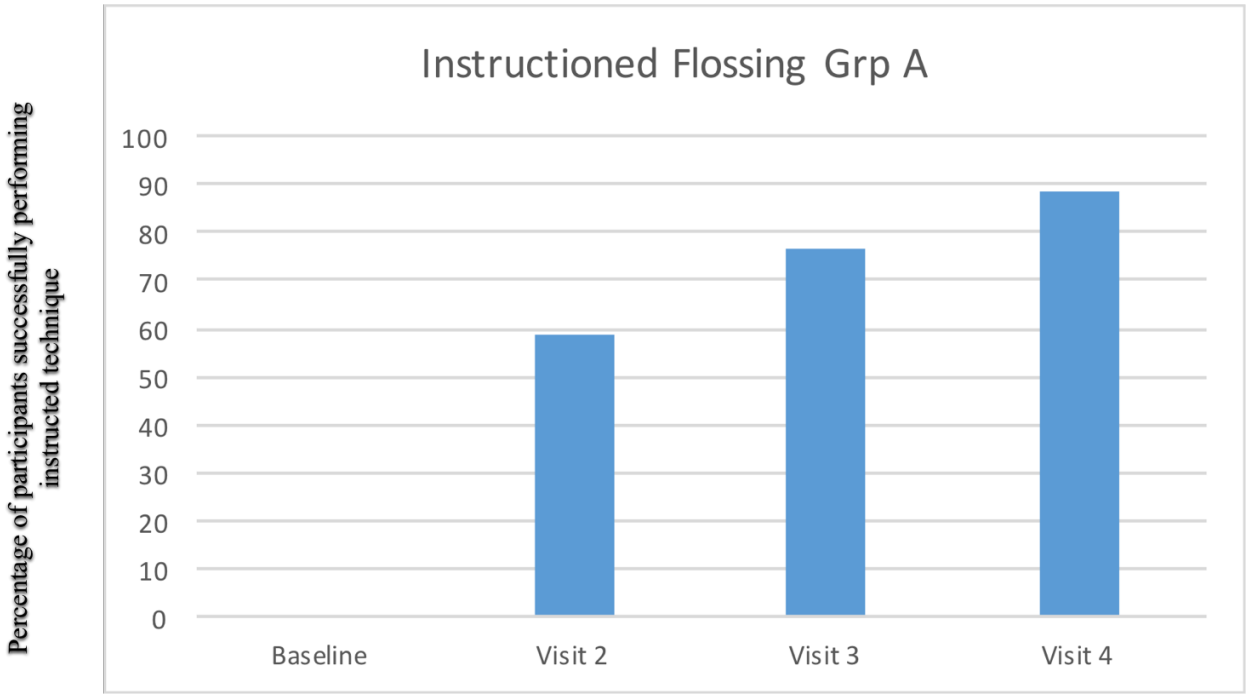


Fig 1 (Percentage of participants in group A successfully the flossing technique instructed)

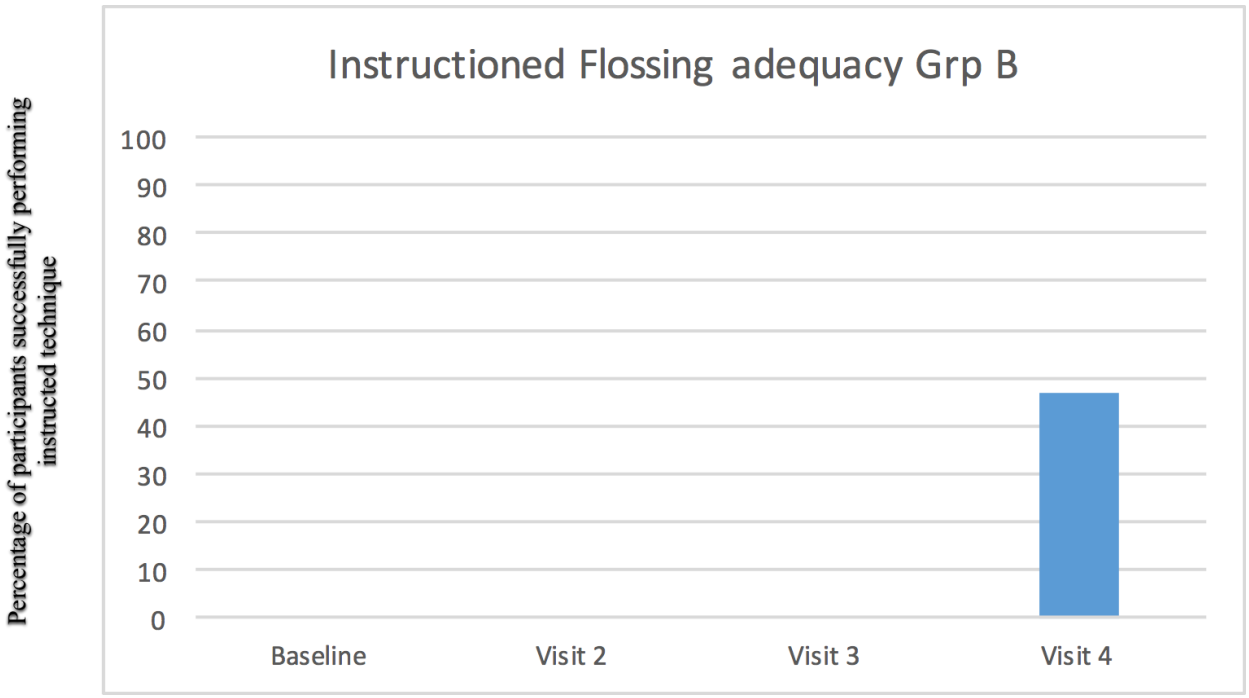


Fig 2 (Percentage of participants in group B successfully the flossing technique instructed)

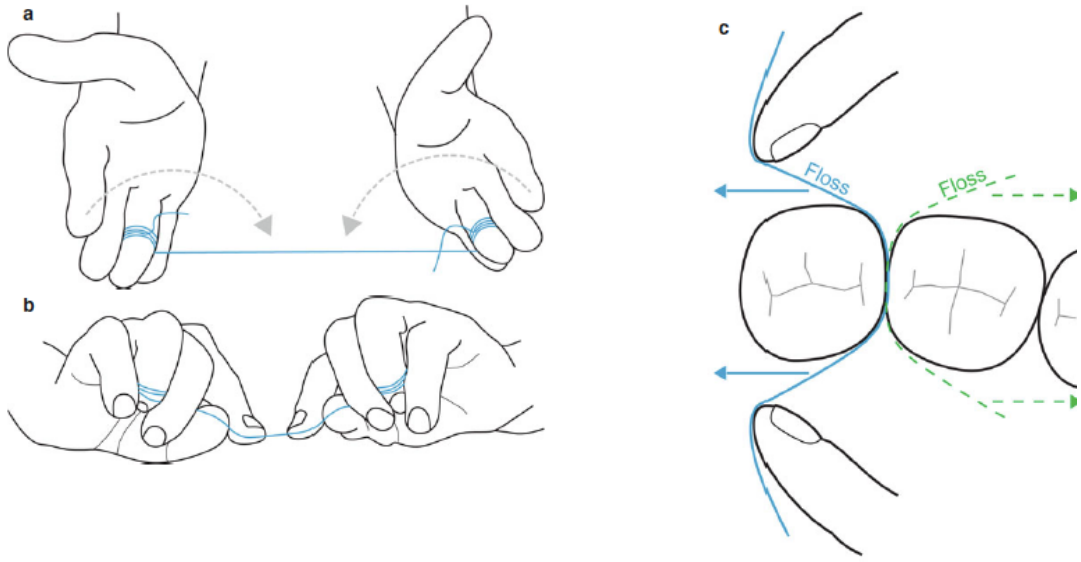


Fig 3 Instructed Dental Flossing Technique

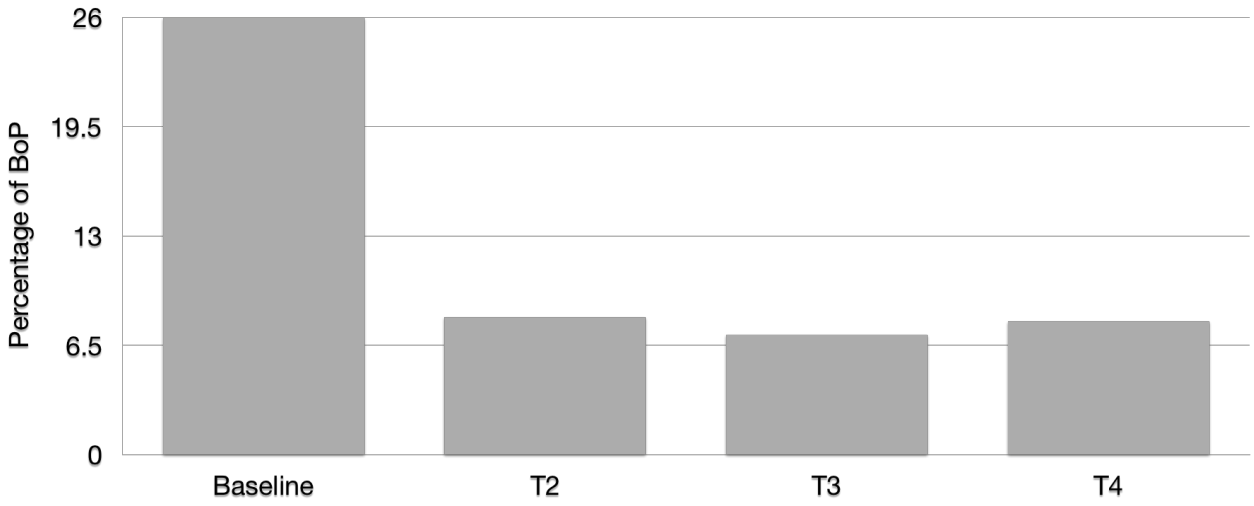


Fig 4 BoP scores Group A

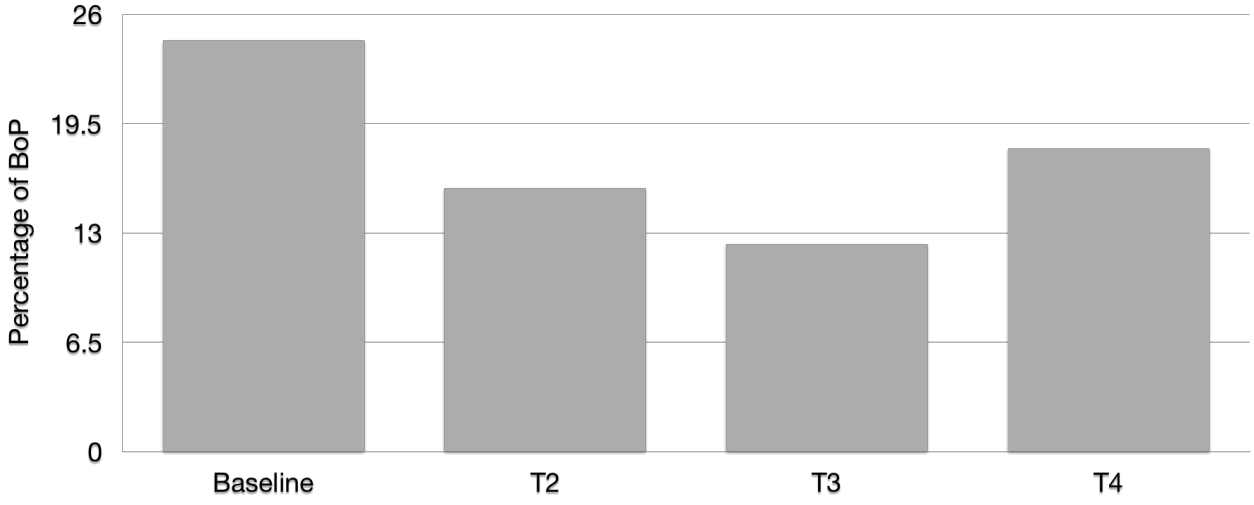


Fig 5 BoP scores Group B

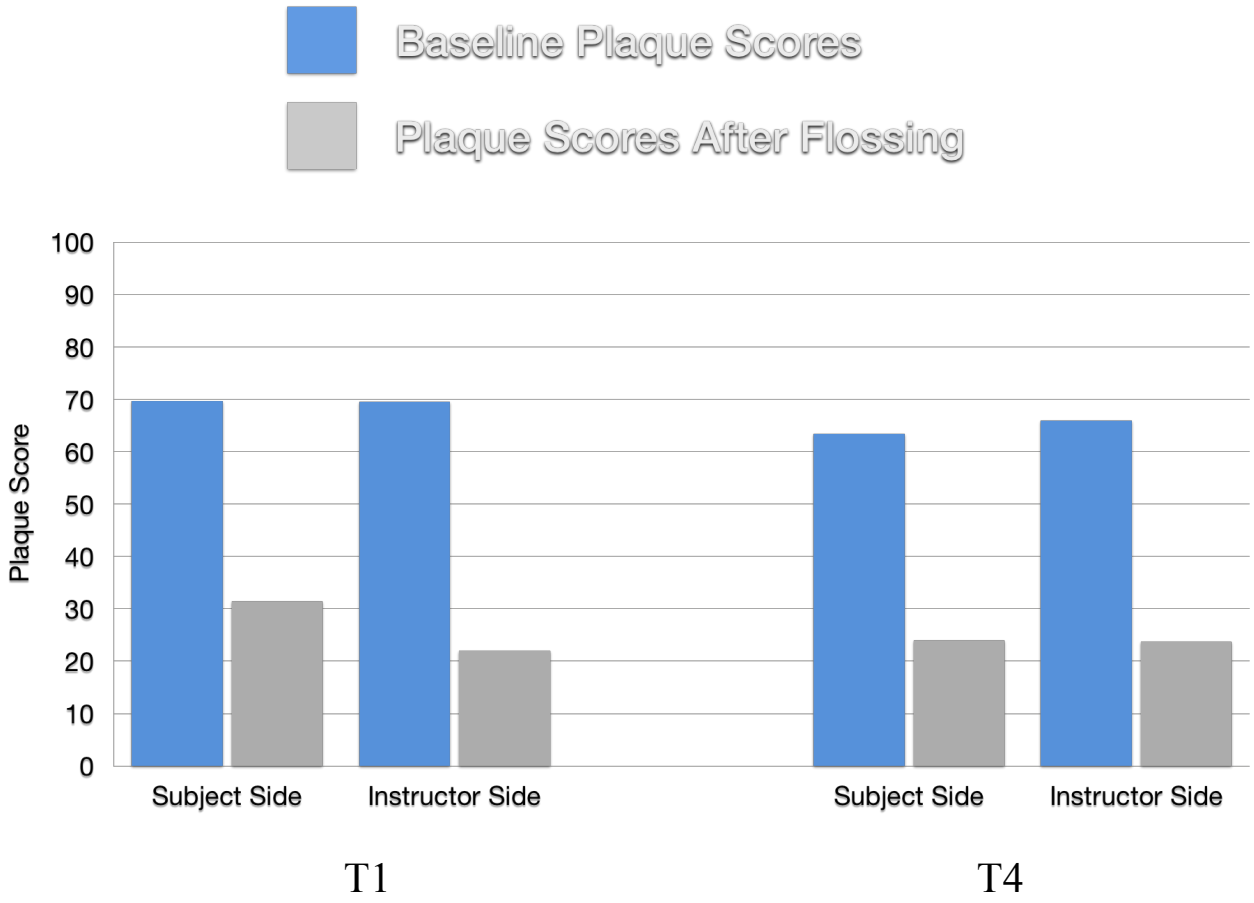


Fig 6: Comparison of reduction in plaque percentage between Visits 1 and 4

Appendix B: Tables

	Group A	Group B	P
Age: Mean (SD)	35.71 (13.32)	32.23 (10.10)	0.398
Gender	6 Males (35.29%)	8 Males (47.06%)	0.486
	11 Females (64.71%)	9 Females (52.94%)	

Table 1: *Baseline Demographics among groups*

First P-value: Independent-samples t-test
Second P-value: Chi-square test

	Baseline vs T2	Baseline vs T3	Baseline vs T4	T2 vs T3	T2 vs T4	T3 vs T4
P	*<0.001*	*<0.001*	*<0.001*	0.250	0.062	0.500

Table 2: *Group A flossing instructions successful performance*

McNemar's test with Bonferroni correction (P< 0.0083)

	Baseline vs T2	Baseline vs T3	Baseline vs T4	T2 vs T3	T2 vs T4	T3 vs T4
P	1	1	*0.007*	1	*0.007*	*0.007*

Table 3: *Group B flossing instructions successful performance*

McNemar's test with Bonferroni correction (P< 0.0083)

	Group A	Group B
Mean	25.98	24.45
Median	26.04	21.43
SD	4.65	8.49
IQR	8.93	2.08
P	0.208	

Table 4: *BoP baseline score*

Mann-Whitney U test

	Mean	Median	SD	IQR	P
Baseline	25.98	26.04	4.65	8.93	<0.001
Visit 2	8.18	6.25	7.37	7.81	
Visit 3	7.13	5.36	5.36	6.86	
Visit 4	7.91	4.81	5.80	7.15	

Table 5: *BoP Group A over the different visits*

Friedman test

	T2 VsT1	T3 Vs T1	T4 VsT1	T3 VsT2	T4 Vs T2	T4 VsT3
P	0.002	<0.001	<0.001	0.49	0.977	0.408

Table 6: *BoP Group A over the different visits*

Wilcoxon signed rank test with Bonferroni correction (P< 0.0083)

	Mean	Median	SD	IQR	P
Baseline	24.45	21.43	8.49	2.08	<0.001
Visit 2	15.65	12.50	12.42	9.26	
Visit 3	12.34	8.33	12.18	6.95	
Visit 4	18.01	13.39	13.15	13.62	

Table 7: *BoP Group B over the different visits*

Friedman test

	T1 Vs T2	T1 Vs T3	T1 Vs T4	T2 Vs T3	T2 Vs T4	T3 Vs T4
P	0.003	<0.001	0.017	0.027	0.276	0.017

Table 8: *BoP Group B over the different visits*

Wilcoxon signed-rank test with Bonferroni correction (P< 0.0083)

	Mean	Median	SD	IQR	P
Group A V4-V1	18.07	18.75	5.72	6.70	0.034
Group B V4-V1	6.44	8.93	9.93	12.64	<0.001

Table 9: *Difference in BoP reduction between Groups A,B*

First P-value: Independent-samples t-test
Second P-value: ANCOVA with adjusting for age, gender and baseline BoP

	Subject side	Instructor side
Mean	69.70	69.58
Median	67.86	67.86
SD	15.39	17.57
IQR	19.67	24.11
P	0.948	

Table 10: *Plaque baseline scores Group A*

Paired t-test

	Mean	Median	SD	IQR	P
Change T1-Subject side	38.25	35.71	14.30	17.71	0.024
Change T1-Instructor side	47.54	45.83	13.75	20.12	

Table 11: *Plaque Group A- Reduction in Plaque % between subject and instructor side at T1*

Paired t-test

	Subject side	Instructor side
Mean	63.41	65.99
Median	64.29	66.07
SD	16.07	16.53
IQR	20.53	30.36
P	0.323	

Table 12: *Plaque T4 scores Group A*

Paired t-test

	Mean	Median	SD	IQR	P
Change T4- Subject side	39.44	35.42	17.44	23.15	0.276
Change T4- Instructor side	42.25	42.86	14.89	26.64	

Table 13: *Plaque Group A- Reduction in Plaque % between subject and instructor side at T4*

Wilcoxon signed-rank test

	Mean	Median	SD	IQR	P
Change T1-Subject side	38.24	35.71	14.30	17.71	0.722
Change T4-Subject side	39.44	35.42	17.44	23.15	

Table 14: *Plaque Group A- Reduction in Plaque % between subject at T1 and T4*

Wilcoxon signed-rank test

	Mean	Median	SD	IQR	P
Change T1-(Instructor-Subject side)	9.30	8.93	15.41	16.37	0.147
Change T4-(Instructor-Subject side)	2.81	7.14	17.92	22.02	

Table 15: *Plaque Group A scores (instructor % plaque removal - subject's % plaque removal) between T1 and T4*

Paired t-test

	Subject side	Instructor side
Mean	67.18	67.30
Median	75.00	73.21
SD	20.64	18.94
IQR	38.45	33.48
P	0.932	

Table 16: *Plaque baseline scores at time 4 Group B*

Paired t-test

	Mean	Median	SD	IQR	P
Change T4- Subject side	38.00	36.54	11.89	14.14	0.005
Change T4- Instructor side	47.13	50.00	17.60	25.89	

Table 17: *Plaque Group B- Reduction in Plaque % between subject and instructor side at T4*

Paired t-test

Appendix C: Forms

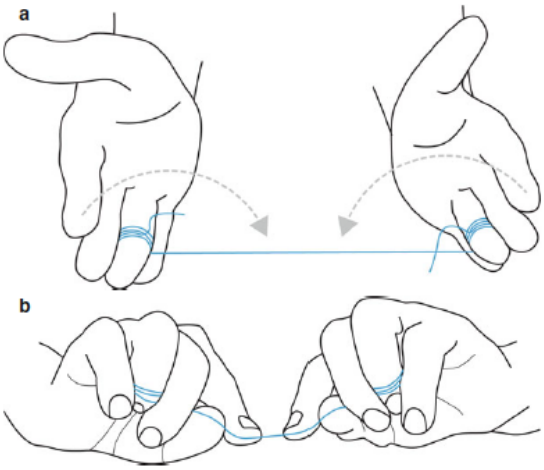
Oral Hygiene Instructions (Group A)

During your participation in this study, please follow these instructions.

Flossing:
Done once a day, with no preference on timing of the day.

Technique:

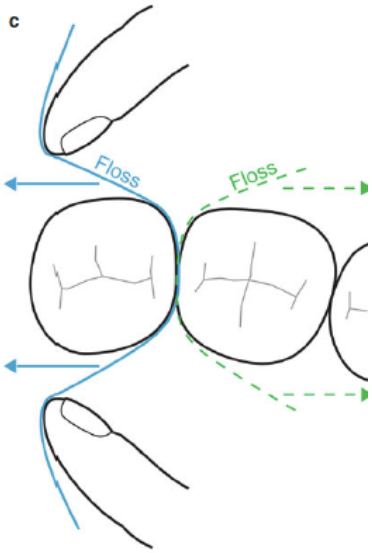
Use an approximate 32 cm of floss/tape (~15–18 inches), which is adapted on the ring (4th fingers of each hand) approximately at the level of the 1st knuckle.



The floss should be adapted enough that when the hands are held with fingers extended and the hands are pulled apart, the floss will be approximately 5–6 inches apart (~10–12 cm).

The floss is then held between the first finger (index finger) and the thumb on each hand.

The floss is controlled by the thumb or index finger to prevent it from cutting into the gingiva.



Heavy pressure through the contact can be reduced by moving the floss in a Forward-Backward direction (sawing) when moving through the contact area

The floss is introduced initially on the end of a last tooth in an arch and tightly adapted to the curved surface of the tooth, like bending a “C” around the tooth.

Next the floss is introduced to the next adjacent area with the same motion, removing plaque from the side surfaces of each tooth,

moving around the arch until reaching the last end of the most posterior tooth on the opposing side of the arch

Lateral force is used and the floss is moved in a short forward-backward motion as well as upwards and downwards motion as if one were drying their back with a towel.

No other interproximal devices are allowed to be used during the total time of the study. For example, proxy brush, water flosser, wooden or plastic toothpick.

No mouth wash is allowed to be used during the total time of the study

Oral Hygiene Instructions (Group B)

During your participation in this study, please keep up with your normal home care.

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