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Toothbrushing and Flossing Sequence: The Most Effective Way to  
Reduce Dental Plaque Biofilm

By

Kimberly Obiedo

B.S., Dental Hygiene, University of New Mexico, 2002

THESIS

Submitted in Partial Fulfillment of the  
Requirements for the Degree of

Master of Science  
Dental Hygiene

The University of New Mexico  
Albuquerque, New Mexico

**July 2024**

## ACKNOWLEDGMENTS

Thank you for your help, Justine Ponce RDH, MS Assistant Professor University of New Mexico Division of Dental Hygiene. Robin Gatlin RDH, MS Assistant Professor University of New Mexico Division of Dental Hygiene. Christine Nathe, RDH, MS Professor, Director University of New Mexico Division of Dental Hygiene.

# **Toothbrushing and Flossing Sequence: The Most Effective Way to Reduce Dental Plaque Biofilm**

**By**

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**M.S., Dental Hygiene, University of New Mexico, 2024**

## **ABSTRACT**

Dental plaque biofilm remaining on oral structures causes disease processes such as gingivitis, periodontitis, and dental caries. This study's purpose was to determine which sequence of dental biofilm removal is more effective, toothbrushing followed by flossing or flossing followed by toothbrushing. The study was conducted using an experimental approach with a split mouth design. A pre and post plaque index score was determined for each participant using Ramfjord's tooth assignment and all data was recorded on a plaque control data sheet. Thirty-two adults were eligible to participate in the study. The average results of the primary plaque score were 78% of tooth surfaces on the right half and 74% on the left half that contained plaque biofilm. Results of the second score were 32% of surfaces on the right side and 35% on the left side still contained biofilm. Statistical analysis shows toothbrushing followed by flossing is more effective.

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Table 1: t-Test: Paired Two Sample for Means in pre-and post-plaque scores on the right and left sides with an alpha value of 0.05.

## *Chapter I: Introduction*

### **Introduction**

Daily oral hygiene recommendations include toothbrushing and flossing since these behaviors remove dental plaque biofilm on teeth surfaces. Dental plaque biofilm left on teeth can result in the demineralization of tooth surfaces and contribute to gingival and periodontium infections. Gingivitis is reversible with proper oral care but when dental plaque accumulates and is left undisturbed, periodontium infection occurs which can cause bone loss. Since toothbrushing alone cannot adequately reach interproximal areas, flossing helps to remove dental plaque biofilm.

### **Statement of the problem**

Does the sequence of toothbrushing and flossing affect the amount of total biofilm removal? Is toothbrushing more effective in removing dental plaque biofilm when done first if compared to dental flossing done first?

### **Significance of the Problem**

Dental plaque biofilm is an organized accumulation of microbial communities that accumulate on tooth surfaces. The biofilm bacteria protect the communities from the washing mechanism of saliva, crevicular fluids, chemotherapeutics, antibiotics, and antiseptics. Dental plaque biofilm protects the community of microorganisms by producing a matrix that encapsulates them with an extra-cellular polymeric substance. The matrix is composed of polysaccharides, proteins, and other compounds. The protective encapsulated matrix easily adheres to the protective pellicle that covers the tooth surface and aids in biofilm adherence. This protective mechanism increases the likelihood of the colonies' survival. While dental plaque biofilm protects itself from the surrounding environment, it cannot protect itself from mechanical methods of removal. Mechanical methods for removal of dental plaque biofilm include toothbrushing, interdental cleaning tools (floss), and professional scaling. All methods effectively disrupt and remove the protective dental plaque biofilm.<sup>1</sup> Toothbrushing is successful in removing dental plaque biofilm on the buccal, lingual, and occlusal surfaces of teeth, also the tongue and oral mucosa. Interdental cleaning is essential for removal of interproximal dental plaque biofilm. Professional dental scaling is also needed regularly for

effective removal of dental plaque biofilm and calculus (mineralized dental plaque biofilm).  
2,3,4

Dental plaque biofilm is the cause of dental caries, gingivitis, and periodontitis.<sup>1,5</sup> Gingival changes appear when dental plaque biofilm matures and proliferate soluble compounds produced by pathogenic bacteria and these penetrate the sulcular epithelium. The soluble compounds stimulate the host cells to produce chemical mediators associated with the inflammatory process. This inflammatory process can either breakdown the collagen and accumulation of an inflammatory infiltrate which leads to clinical signs of gingivitis, or it will breakdown the collagen in the periodontal ligament and cause resorption of the supporting alveolar bone resulting in periodontitis, or bone loss.<sup>1</sup> Dental plaque biofilm is considered mature when it is left undisturbed for 72 hours.<sup>23</sup> Changes in the hard tissue of the tooth or enamel is called dental caries or a carious lesion. Carious lesions develop when dental plaque biofilms mature and remain on tooth surfaces for extended periods of time, resulting in demineralization. Demineralization is the process of minerals in the tooth structure being dissolved by acids formed from dental plaque biofilm metabolizing fermentable carbohydrates.<sup>6,23</sup>

The inflammatory process that creates periodontitis is associated with systemic diseases and overall health. The dental plaque biofilm creates a breakdown of the epithelial tissue of the periodontium. This breakdown of the protective (barrier) epithelium is thought to allow bacterial biofilm to enter the body through the circulatory system and potentially contribute to systemic inflammation. Periodontitis is associated with systemic diseases like cardiovascular disease, diabetes mellitus, respiratory disease, adverse pregnancy outcomes, obesity, pancreatic cancer, and Alzheimer's disease. Effective removal of bacterial plaque biofilm can improve our patient's overall health.<sup>1</sup>

### **Operational Definitions**

Toothbrushing- the act of removing biofilm from the tooth surface with a toothbrush.

Flossing- removing biofilm from interproximal tooth surfaces with a floss thread.

Biofilm- a matrix composed of eDNA, proteins and polysaccharides with a high resistance to antibiotics.

Periodontitis- irreversible infection within the supporting tissues of the tooth and destruction of supporting bone.

Gingivitis- reversible inflammation of the gingival tissue.

Dental caries – localized demineralization of tooth surfaces.

## *Chapter II: Review of Literature*

### **Introduction**

The review of literature focuses on toothbrushing and flossing and their association with gingival health. Medical and dental literature was reviewed using PubMed/MeSH search engines. Key search words used were “toothbrushing,” “flossing,” “oral health” and “gingival disease.” General information regarding toothbrushing, flossing and gingival disease was discussed. Gingival disease causes and effects will be reviewed.

### **Toothbrushing**

Toothbrushing mechanically removes dental plaque biofilm from the oral cavity. Regular daily toothbrushing is recommended for the removal of dental plaque biofilm to prevent gingivitis, periodontitis, and dental caries. The American Dental Association (ADA) recommends that individuals brush their teeth for two minutes twice a day. Brushing twice daily has been shown to effectively remove dental plaque biofilm.<sup>7,8</sup> There are several techniques that can be suggested depending on the patient’s oral condition, but the focus should be that all surfaces of the tooth that can be accessed with a toothbrush are cleaned; gingival injury should be avoided by using a soft bristle toothbrush and light pressure.<sup>8,9,10</sup> In general, all methods are similar or modifications of the other. The ADA suggests that the toothbrush be placed at a 45- degree angle toward the gingival margin to remove biofilm from just above and below the gingival margin. The toothbrush should be gently moved in a back-and-forth motion. The toothbrush should be placed vertically using up and down strokes for the lingual surfaces of anterior teeth and in a back-and-forth motion on the occlusal surfaces.

One study suggests that the Modified Bass technique is the most common method recommended for adults and the Scrub and Fones technique was the most frequently suggested for children.<sup>11</sup> The Modified Bass Technique is when the toothbrush bristles are placed slightly under the gingal margin at a 45-degree angle and small horizontal strokes, or a circular motion are used. The toothbrush must be placed vertically for anterior teeth using a back-and-forth motion. The occlusal surfaces of teeth will be brushed with the toothbrush bristles placed on top of the occlusal surface using a back-and-forth motion. The toothbrush

is gently moved back and forth or in tiny circles until all teeth are cleaned and every surface of the tooth is brushed.

The Fones technique is the original toothbrushing method. It was recommended by Dr. Fones, the founder of dental hygiene. This technique has been around since 1913 and is suggested for children because it is easy, however, it is less effective than other techniques. The Fones method of toothbrushing is achieved when the maxillary and mandibular teeth are occluded, and the toothbrush is placed against the teeth and is moved in a large circular motion.<sup>12, 13</sup> In the Scrub technique the toothbrush bristles are placed at a right angle to the long axis of the teeth and a horizontal scrubbing action is performed. This technique is simple but can cause cervical abrasion of the tooth. Supragingival dental plaque biofilm is easily removed from the smooth surfaces of teeth, but it is much harder to access in protected areas such as cracks, fissures, and tight spaces between the teeth.

### **Interproximal Cleaning**

Flossing, like toothbrushing, is a method useful for the mechanical removal of dental plaque biofilm. Floss is a type of interproximal cleaner. Interproximal cleaners help to remove debris and dental plaque biofilm from in between the teeth. The interproximal areas between teeth and at the gingival margin are hard to reach areas and thus are prone to gingival disease and interproximal decay. Daily flossing significantly reduces the amount of plaque found between the teeth compared to manual toothbrushing alone.<sup>14, 15</sup>

There are several types of interproximal cleaners like interdental brushes, toothpicks, and waterjet devices.<sup>16</sup> The proper flossing technique according to the American Dental Hygiene Association (ADHA) is to wind 18" of floss around the middle fingers of each hand. Pinch the floss between the thumbs and index fingers leaving a 1"-2" length in between. Keep the floss taut between fingers and gently guide floss in a zig zag motion between the teeth to break between the contact. Gently wrap the floss in a C-shape around one tooth and move the floss in an up and downward motion against the side of one tooth and repeat on the other tooth, being sure to place the floss under the gingival margin. Use the thumbs to direct the floss between all maxillary teeth and the index fingers to glide the floss between the contacts of the mandibular teeth. It is recommended that flossing be done at least once a day.<sup>4,5</sup>

## **Periodontal Health**

The definition of oral health is “being free from chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft palate, periodontal disease, tooth decay and tooth loss, and other diseases and disorders that affect the oral cavity.”<sup>17</sup> It is recommended that individuals spend 5 to 6 minutes per day to maintain healthy teeth and gingiva. The literature tells us that meticulous dental plaque biofilm removal with a toothbrush and an interdental cleaning device is adequate to prevent gingivitis.<sup>18</sup> Dental plaque biofilms play a significant role in the development and pathogenesis of caries, gingivitis, and periodontitis.

Periodontal disease affects half (50%) of the adult population. The prevalence of periodontitis increases as individuals age with 75% of all adults over the age of 65 exhibiting signs of periodontitis. The development of periodontal disease is also associated with genetics and risk factors such as smoking, type I and II diabetes, stress, cardiovascular disease, and obesity.<sup>20,21</sup> This shows a direct correlation between oral health and overall health.

## **Dental Plaque Biofilm**

Dental plaque biofilm forms on all surfaces throughout the mouth including soft tissues and artificial materials such as dentures and implants. Undisturbed dental plaque biofilm accumulates at the gingival margins and begins to affect the host. When the dental plaque biofilm is left undisturbed it initiates a localized inflammatory response resulting in red and bleeding gums also known as gingivitis. Gingivitis is associated with an increase in bacterial species of *Fusobacterium*, *Lachnospiraceae*, *Lautropia* and *Prevotella*. Gingivitis is reversible with the removal of dental plaque biofilm. When dental plaque biofilm is effectively removed the tissues become healthy again. The tissue is pale pink and firm associated with dental health and the biofilm found is *Rothia dentocariosa*.

Subgingival dental plaque biofilms that are left undisturbed can trigger a more aggressive immune response leading to a gradual destruction of the gingival tissues supporting the teeth. This destruction is known as periodontitis. Subgingival dental plaque biofilm associated with chronic periodontitis tends to be enriched in microorganisms such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *Filifactor alocis*, *Treponema denticola* and *Fretibacterium sp.*<sup>19</sup>.

Dental plaque biofilm cannot be eliminated but with meticulous oral hygiene it can be reduced, and oral health maintained. Maintenance of healthy bacterial flora in the oral cavity can be done in 5 to 6 minutes a day by proper toothbrushing twice daily and flossing once a day. Proper oral hygiene can result in the prevention or management of periodontal disease and the impact that periodontal disease may have on systemic disease<sup>22</sup>.

### **Summary**

Toothbrushing techniques are similar. Correct toothbrushing effectively removes dental plaque biofilm from smooth surfaces of the tooth. Areas of the tooth that are difficult to reach, like interproximal areas, require interproximal cleaning agents like floss to effectively remove dental plaque biofilm. This can be done by toothbrushing two times a day and flossing once a day for a combination of 5-6 minutes a day.

Effective removal of dental plaque biofilm results in a decrease in gingivitis, periodontal disease, and tooth decay. This will improve oral health and overall systemic health. Results of the most effective technique for removing plaque biofilm will help dental hygienists better educate their patients.



### *Chapter III: Methods and Materials*

#### **Introduction**

This research study investigated the effectiveness of toothbrushing and flossing sequences on dental plaque removal from tooth surfaces. The aim was to determine if it is more effective to brush first, then floss or floss first and then brush. Previous studies have focused on the effectiveness of toothbrushing and flossing in plaque biofilm removal, but few have focused on sequence effectiveness. This study evaluated biofilm removal of participants utilizing a plaque control index, taken before and after, when participants brushed and then flossed (method A) and then flossed and brushed (method B). The study was conducted through an experimental approach using a dental hygiene provider, study participants, a mirror, and a chewable disclosing tablet. The plaque indices were analyzed and were compared on the study subjects to determine which sequence was more effective in removing dental plaque biofilm.

#### **Sample Description**

The sample population for this study consisted of a convenience sample from the Department of Dental Medicine from the University of New Mexico. The target population consisted of subjects 18 and older, with varying gender and races.

#### **Research design**

Utilizing the convenience sample, a recruitment email was sent to the prospective participants through the Department of Dental Medicine Email Listserv's. The participants were briefed on the study in the email and had a copy of the detailed consent to read ahead of time. Prospective participants were invited to participate voluntarily in the toothbrushing and flossing sequence study. The prospective participants were notified at 2 weeks, 1 week and one day prior to the study date. A condition of enrollment included participants being asked not to brush or floss their teeth for a minimum of 12 hours prior to their participation in the study and participants needed to be screened for eligibility of contacting teeth with the Ramfjord's tooth assignment (teeth numbers 3, 9, 12, 19, 25, 28). Informed consent detailing the study was collected the day of. This study took place in an established dental hygiene educational setting at the University of New Mexico HSC (Health Sciences Center) North Campus in Novitski Dental Hygiene Clinic.

This experimental study was conducted using a dental hygiene provider, study subjects, a dental mirror, a chewable disclosing tablet, floss, and a toothbrush. The two independent variables in this study are floss and a soft bristle toothbrush. This study used a split mouth design. On the right side of the mouth, Method (A), the teeth will be brushed first for 1-minute or 30-seconds per arch, then flossed. On the left side of the mouth, Method (B), the teeth were flossed followed by a 1-minute brush or 30-seconds per arch.

To begin the study, the participant chewed on a disclosing tablet, spit, and rinsed with water. Then a dental hygiene provider determined and scored a plaque index on the subject using a Plaque Control Record (PCR) equation by evaluating the Ramfjord's tooth assignments. The disclosing tablet worked by the disclosing solution adhering to areas where plaque is present on tooth surfaces. Those areas were highlighted with color from the disclosing solution. A PCR was then calculated by counting the number of areas that show plaque on them divided by the number of total surfaces possible. The subjects had a pre-PCR recorded which was prior to any oral. Hygiene techniques, and a post-PCR recorded after completing the tasks.

### **Data Collection and Analysis**

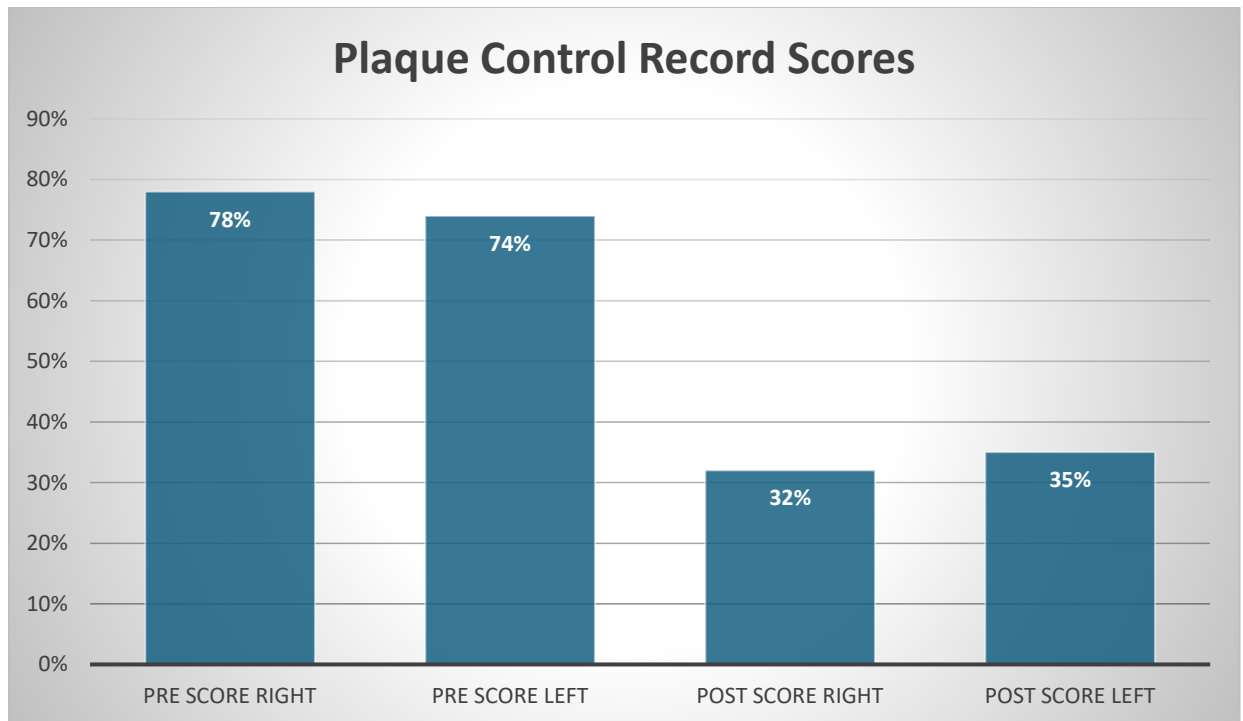
Data from the study was recorded using the PCR data collection sheet. The collection sheet recorded the pre- and post-plaque score for both right and left sides. The data was then analyzed through descriptive statistics and testing for comparing the pre- versus the post-plaque scores between Method A and Method B. A two-sample paired t-test with a means alpha of 0.05 was carried out on the data along with correlation testing from a Pearson's correlation. Additionally, a calculated t-statistic of 2.741 is also larger than the critical value which suggests that the difference between the two methods is statistically significant.

## *Chapter IV: Results, Discussion, and Conclusion*

### **Results**

Thirty-two adults were eligible to participate in the study. The average results of the primary plaque score were 78% of tooth surfaces on the right half (Method A) of the mouth and 74% on the left half (Method B) of the mouth that contained plaque biofilm (Figure 1).

After determining a primary plaque index score, and participating in the brushing and flossing activity, another plaque index score was obtained. The second plaque index score was determined on participants using the same teeth from Ramfjord's tooth assignment as used in the primary plaque index score. The results of the second plaque index score were an average of 32% of surfaces on participants right half (Method A) and 35% of surfaces on the participants left half (Method B) of the mouth had residual plaque biofilm (Figure 1). Pre-score plaque biofilm values were higher than post-score as expected.



*Figure 1 Average plaque control record scores of pre-and post-examinations for right side (Method A) and left side (Method B)*

The data from this study shows that Method A which was brushing first then followed by flossing resulted in a lower plaque score. Figure 2 illustrates this data side by side with comparison of pre- and post- plaque index scores.

The pre-score plaque biofilm scores for both sides of the mouth ranged from 100% to 25%. When comparing the pre- and post- averages, the mean post-treatment plaque biofilm score on the right was 46% with a standard error of 0.043 and a standard deviation of 0.24 while the post-score value on the left was 37% with a standard error of 0.040 and a standard deviation of 0.22. The median pre-score was 42% while the post-score value of 34%. The mode pre-score value was 33% and the post-score was 50%. These results are shown in Figure 2.

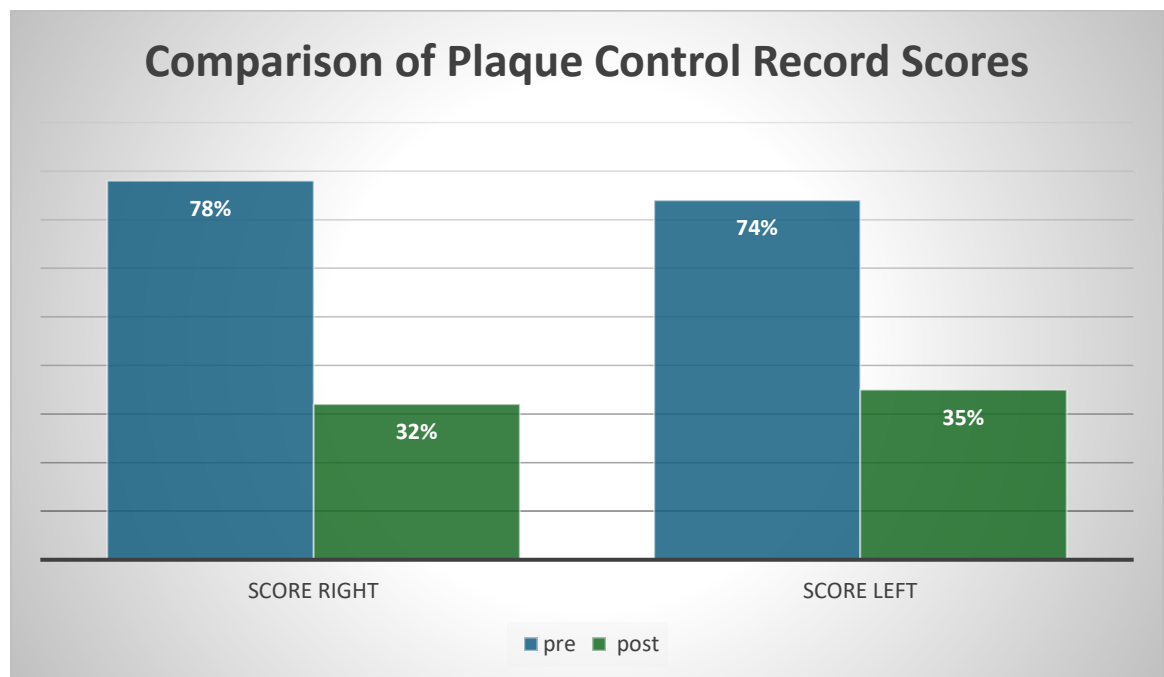


Figure 2: Average plaque control record scores for pre-and-post examination shown side by side.

To determine if the difference between the mean change in Method (A) and Method (B) was statistically significant data analysis was done. This was done using a two-sample paired t-test with a means alpha of 0.05. Results show a 5.7% variance from the mean change in scores on the right side and a 5.1% variance in change in scores on the left side. The low variance shows that measures in each group do not significantly vary from the mean. The correlation shows a Pearson's correlation measure of 0.732 which illustrates a moderate

positive correlation between the two treatment conditions. Statistical significance is illustrated by a one-tailed p-value of 0.005 and a two-tailed p-value of 0.010 in the t statistic. The calculated t-statistic of 2.741 is also larger than the critical value which suggests that the difference between the two methods is statistically significant. Statistical analysis shows Method (A) toothbrushing then flossing is more effective than Method (B) flossing followed by toothbrushing at removing dental plaque biofilm. (Table 1)

Table 1: t-Test: Paired Two Sample for Means in pre-and post-plaque scores on the right and left sides with an alpha value of 0.05.

<i>t-Test: Paired Two Sample for Means</i>		
	<i>Change in scores Right</i>	<i>Change in scores Left</i>
<i>Mean</i>	0.457096774	0.372580645
<i>Variance</i>	0.057947957	0.051633118
<i>Observations</i>	31	31
<i>Pearson Correlation</i>	0.732323925	
<i>Hypothesized Mean Difference</i>	0	
<i>df</i>	30	
<i>t Stat</i>	2.741339749	
<i>P(T&lt;=t) one-tail</i>	0.005106063	
<i>t Critical one-tail</i>	1.697260887	
<i>P(T&lt;=t) two-tail</i>	0.010212126	
<i>t Critical two-tail</i>	2.042272456	

## Discussion

The number of participants who participated in the study was limited (N = 32). Toothbrushing is successful in removing dental plaque biofilm on the buccal, lingual, and occlusal surfaces of teeth, also the tongue and oral mucosa. Interdental cleaning is essential for removal of interproximal dental plaque biofilm.<sup>2,3,4</sup> Toothbrushing and flossing combined effectively disrupt and remove dental plaque biofilm.<sup>1</sup> If left undisturbed, plaque biofilm can affect the oral cavity by causing dental caries, gingivitis, and periodontitis.<sup>1,5</sup>

The exploration aimed to investigate whether brushing teeth before flossing or flossing before brushing is more effective in managing dental plaque biofilm. Experimental conditions were differentiated by having a Method A and Method B study design. The results show a one-tailed p-value: 0.005 and two-tailed 0.010 which means that there is a significant difference in

plaque biofilm reduction between brushing first then flossing (Method A) and flossing first then brushing (Method B) methods. (Table 1).

Although, after a review of the literature, there were minimal to no studies evaluating the most effective sequence for biofilm removal and one study determined the opposite results. Toothbrushing followed by flossing or flossing followed by toothbrushing. One study from Mazhari et., al, which was a randomized controlled crossover trial discovered that flossing followed by toothbrushing was more effective than toothbrushing followed by flossing.<sup>24</sup> It is important to acknowledge, the findings from this study add to the large volume of literature on plaque management through brushing and flossing teeth.

### **Limitations**

Limitations to this study include different pressure applied while toothbrushing by participants. The suggested pressure for removal of dental plaque biofilm is less than 400 g (about half the weight of a water bottle) of pressure.<sup>23</sup> Increased pressure effectively removes more dental plaque biofilm. Participants that apply heavier pressure might effectively remove more dental plaque biofilm than those that place lighter pressure. If participants apply different amounts of pressure, it can affect the results of post-remaining plaque biofilm results.

Oral hygiene instruction was not specified to participants before toothbrushing and flossing were performed. There are different techniques recommended by dental professionals to use while toothbrushing for the effective removal of dental plaque biofilm. Flossing should be done with a c-shape method to effectively remove dental plaque biofilm. The result of participants not being given instruction on effective techniques for the removal of dental plaque biofilm means they may have used different techniques or a lack of technique for effective biofilm removal.

Participants that have teeth with crowns were not excluded from the study. Tooth enamel is textured and therefore dental plaque biofilm and disclosing tablet dyes adhere to enamel easier than the smooth surface of crowns.

However, this study is also limited by the small sample which limits generalizability. The assessment was also based on short-term brushing and flossing events which overlooks

long-term effects. The method used, the Ramfjord's tooth index, is also limited to visual inspection which introduces subjectivity in measurements.

### **Conclusion**

This experimental approach study's purpose was to determine which method toothbrushing followed by flossing or flossing followed by toothbrushing would be more effective in removing dental plaque biofilm. The hypothesis of the study was toothbrushing followed by flossing would be more effective in removing dental plaque biofilm. The results from the study indicated by a significantly statistical difference that toothbrushing before flossing is more effective than flossing followed by toothbrushing.

### **Recommendations for Further Research**

Future exploration should include research on the effectiveness of the sequence of toothbrushing and flossing would be to provide oral hygiene instruction to participants before they perform toothbrushing and flossing. Oral hygiene instruction would ensure participants use the same technique and appropriate pressure to effectively remove dental plaque biofilm. Providing instruction on proper technique would improve reliability of the study.

Another suggestion would be to eliminate participants that have crowns and other restorations on the required teeth to participate. Dental materials do not have the same surface that dental enamel has and could limit the amount of dental plaque biofilm that is available for effective research for the study. Dental materials can also cause improper contacts between teeth affecting efficacy of flossing.

Lastly, increasing the number of participants would increase the sample size. Increasing the sample size would give a better representation of the population and provide more accurate results for the study. The result could be a larger difference in the averages of the two sequences.

## *Chapter V: Article for Submission*

### ABSTRACT

**Purpose:** This study's purpose was to determine which sequence of dental biofilm removal is more effective, toothbrushing followed by flossing or flossing followed by toothbrushing.

**Methods:** The study was conducted using an experimental approach which included a dental hygiene provider, study subjects, dental mirror, and a disclosing tablet. The two independent variables in this study are floss and a soft bristle toothbrush. This study used a split mouth design. A pre and post plaque index score was determined for each participant using Ramfjord's tooth assignment and all data was recorded on a plaque control data sheet.

**Results:** Thirty-two adults (N=32) consented to participate in the study. Plaque index scores were determined using Ramfjord's tooth assignment with four surfaces for biofilm retention (N=12). A primary plaque score was determined on each participant. The pre-score plaque biofilm scores for both sides of the mouth ranged from 100% to 25%. The results of the second plaque score were 32% of surfaces on the right side of the mouth and 35% on the left side still contained biofilm. Pearson's correlation measure of 0.732 which illustrates a moderate positive correlation between the two treatment conditions. Statistical significance is illustrated by a one-tailed p-value of 0.005 and a two-tailed p-value of 0.010 in the t statistic which means that there is a significant difference in plaque biofilm reduction between brushing first then flossing and flossing first then brushing methods.

**Conclusion:** Dental plaque biofilm remaining on oral structures causes disease processes such as gingivitis, periodontitis, and decay. There is a lack of research for dental professionals to refer to when recommending oral hygiene sequence to patients. The results of the study indicate by a small percentage that toothbrushing prior to flossing is more effective in removing more dental plaque biofilm.



## Introduction

Dental plaque biofilm is the cause of dental caries, gingivitis, and periodontitis.<sup>1,5</sup> Gingival changes appear when dental plaque biofilm mature and proliferate soluble compounds produced by pathogenic bacteria and these penetrate the sulcular epithelium.<sup>1</sup> The result is gingivitis and periodontitis. Allowing biofilm to mature and remain on tooth surfaces also causes the development of demineralized areas. Demineralization is the process of minerals in the tooth structure being dissolved by acids formed from dental plaque biofilm metabolizing fermentable carbohydrates.<sup>6,23</sup> Cavities begin as demineralization.

A healthy oral cavity is “being free from chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft palate, periodontal disease, tooth decay and tooth loss, and other diseases and disorders that affect the oral cavity.”<sup>17</sup> As dental providers our goal is dental health for our patients. Oral health providers instruct patients on techniques to achieve oral health. It is recommended that individuals spend 5 to 6 minutes per day to maintain healthy teeth and gingiva. The literature established that meticulous dental plaque biofilm removal with a toothbrush and an interdental cleaning device is adequate to prevent gingivitis<sup>18</sup>. Biofilm maturation is significant in the development of gingivitis, periodontitis, and decay and needs to be effectively removed.

The literature established toothbrushing mechanically removes dental plaque biofilm from the oral cavity. Regular daily toothbrushing for two minutes two times a day is recommended for effectively removing dental plaque biofilm for preventing gingivitis, periodontitis, and dental caries.<sup>5</sup> Toothbrushing alone is not effective in removing biofilm. Daily flossing the hard-to-reach interproximal areas between teeth and gingival margin significantly reduces biofilm in the areas prone to gingival disease and interproximal decay.<sup>14,15</sup>

Effectively removing biofilm results in a decrease in gingivitis, periodontitis, and tooth decay. For patients to achieve dental health, dental professionals should have knowledge of the best techniques and practices to share with patients. After a review of the literature, there were minimal to no studies evaluating the sequence of toothbrushing and flossing. This study focused on toothbrushing and flossing sequence; to investigate if it is more effective to brush first, then floss or floss first and then brush. Previous studies focused

on the effectiveness of toothbrushing and flossing in plaque biofilm removal, but few have focused on sequence effectiveness.

## **Methods**

The study's aim was to evaluate biofilm removal of participants using a plaque score index taken before and after participants brushed then flossed the right half of the mouth, or flossed then brushed the left half of their mouth. A plaque score index is determined by first breaking down the tooth into four surfaces mesial, distal, facial, or lingual. Teeth were chosen using Ramfjord's tooth assignment. Each tooth has 4 surfaces and 3 teeth per half of the mouth, with 12 surfaces to contain biofilm. The surfaces containing dyed plaque biofilm (N) were counted per side and divided by total surfaces  $N/12 = \text{surfaces with plaque biofilm}\%$ .

The experimental approach research study was conducted using a dental hygiene provider, 32 adult participants, a mirror, a disclosing tablet, floss, and soft bristled toothbrush. Participants performed toothbrushing followed by flossing (Method A) on the right side of the mouth and flossing then toothbrushing (Method B) on the left side of the mouth. The University of New Mexico's IRB reviewed and approved the study. Two weeks before the date of the study, an email was sent to recruit prospective participants from the Department of Dental Medicine via Listserv. *One week before and one day before the study* a reminder email was sent to participants with instructions not to brush or floss their teeth for 12 hours before the study.

Upon arrival, participants were visually examined to confirm eligibility. Eligible study participants are required to have the maxillary right first molar, maxillary left central incisor, maxillary left first bicuspid, mandibular left first molar, mandibular right central incisor, and mandibular right first bicuspid teeth with proximal contacting teeth. Thirty-two adults consented to participate in the study. Participants chewed a disclosing tablet completely and swished it for 30 seconds. Participants then spit the disclosing tablet in the sink and rinse their mouth with water. A primary plaque index score was determined using Ramfjord's tooth assignment which are the maxillary right first molar, maxillary left central incisor, maxillary left first bicuspid, mandibular left first molar, mandibular right central incisor, and mandibular right first bicuspid.  $N/12 = \text{surfaces with plaque biofilm}\%$ .

After determining a primary plaque index score, the participants were instructed to brush the right half of their mouth for 1 minute. They were then instructed to floss their entire mouth. Finally, the participants were instructed to brush the left side of their mouth for 1 minute. All participants were timed by a committee member to ensure they spent the correct time allowed on each side of the mouth. Lastly, a second plaque index score was determined on participants using the same teeth from the Ramfjord's tooth assignment.

## **Results**

The result of the primary plaque score was an average of 78% of teeth surfaces on the right half of the mouth contained plaque biofilm and 74% on the left half of the mouth. The plaque score of all 32 participants were combined and divided by the number of participants to determine the pre- and post plaque score for each half.

The results of the post plaque index scores were an average of 32% of surfaces on the right half and 35% of surfaces on the left half of the mouth had retained biofilm. Figure 2 shows the pre- and post-average results of the study.

The study result is, the right half (Method A) which the participants brushed for a minute then flossed, a difference of 46% biofilm removal was noted from pre- and post-plaque scores. The left side (Method B), in which participants flossed then brushed for a minute there was a 39% difference in biofilm removal on surfaces. The study determined a 7% difference in the different sequences. Statistical analysis shows Method (A) toothbrushing then flossing is more effective than Method (B) flossing followed by toothbrushing at removing dental plaque biofilm.

## **Discussion**

Toothbrushing is successful in removing biofilm on the buccal, lingual, and occlusal surfaces of teeth, also the tongue and oral mucosa. Interdental cleaning is essential for removal of interproximal biofilm.<sup>2,3,4</sup> Toothbrushing and flossing combined effectively disrupt and remove biofilm.<sup>1</sup> If left undisturbed, dental plaque biofilm mature and affect the oral cavity by causing dental caries, gingivitis, and periodontitis.<sup>1,5</sup>

After a review of the literature, there is a significant amount of research on the efficacy of toothbrushing and flossing but, there are minimal studies to determine if sequence

of toothbrushing and flossing affects biofilm removal. A randomized controlled crossover study used 25 dental students over a two-week period. The participants brushed then flossed for one week and the next week they flossed then brushed.<sup>24</sup> The result from the study determined flossing followed by toothbrushing was more effective than toothbrushing followed by flossing.<sup>24</sup>

This study was conducted using an experimental approach to determine which sequence toothbrushing followed by flossing or flossing followed by toothbrushing would be more effective in the removal of dental plaque biofilm. Limitations to the study include different pressure applied while toothbrushing by participants. Oral hygiene instruction was not specified to participants before toothbrushing and flossing were performed. All participants should use the same technique when toothbrushing and flossing. Participants that have teeth with crowns or restorations were not excluded from the study. Study sample size should be increased to have a better representation of the population.

### **Conclusion**

This study's purpose was to determine which sequence toothbrushing followed by flossing or flossing followed by toothbrushing would be more effective in removing biofilm. The hypothesis stated that toothbrushing followed by flossing would be more effective in removing biofilm. The results from the study supported the hypothesis by a small percentage. There is a limited amount of research related to sequence of toothbrushing and flossing efficacy. Therefore, more research is needed to determine the effective sequence for biofilm removal.

## *APENDICES*

Appendix A: HRPO Approval Letter

Appendix B: Informed Consent

Appendix C: Recruitment Email

Appendix D: HRP-226 DSRF

Appendix E: Data Collection Sheet

## Appendix A: HRPO Approval Letter



### Human Research Protections Program

February 16, 2024  
Justine Ponce  
jstambaugh@salud.unm.edu

Dear Justine Ponce:

On 2/16/2024, the HRRC reviewed the following submission:

Type of Review: Initial Study  
Title of Study: Toothbrushing and Flossing Sequence: The Most Effective  
Way to Reduce Dental Plaque Biofilm  
Investigator: Justine Ponce  
Study ID: 23-167  
Submission ID: 23-167  
IND, IDE, or HDE: None

Submission Summary: Initial Study

Documents Approved:

- 581 UNM Protocol template-V3.pdf
- HRP-507 Template Consent
- Participation recruitment email
- Participation recruitment reminder email 1 day V3.pdf
- Participation recruitment reminder email 1 week V3.pdf
- PCR Data Collection Sheet.pdf

Review Category: EXPEDITED: CATEGORIES (4) Noninvasive procedures

Determinations/Waivers: Students / Employees.  
Requires a signed Consent form.  
HIPAA Authorization Addendum Not Applicable.

Submission Approval Date: 2/16/2024  
Approval End Date: 2/15/2025  
Effective Date: 2/16/2024

The HRRC approved the study from 2/16/2024 to 2/15/2025 inclusive. If modifications were required to secure approval, the effective date will be later than the approval date. The "Effective Date" 2/16/2024 is the date the HRRC approved your modifications and, in all cases, represents the date study activities may begin.

Before 2/15/2025 or within 45 days of study closure, whichever is earlier, you are required to submit a continuing review. You may submit a continuing review by navigating to the active study and clicking the "Create Modification/CR" button.

**The University of New Mexico Health Sciences Center**  
**Consent and Authorization to Participate in a Research Study**  
**Toothbrushing and Flossing Sequence: The Most Effective**  
**Way to Reduce Dental Plaque Biofilm**

You are being invited to take part in a research study surrounding the efficacy of flossing and brushing sequence.

**WHAT IS THE PURPOSE, PROCEDURES, AND DURATION OF THE STUDY?**

The aim of this study is to evaluate biofilm removal from teeth of participants utilizing a plaque index, taken before and after, when participants brush and then floss or floss and then brush. The study will be conducted through an experimental approach using a dental hygiene provider, study subjects, a mirror, and a disclosing agent. Study subjects will use the method (A) of toothbrushing followed by flossing on the right side of the mouth and (B) flossing then toothbrushing on the left side of the mouth. Toothbrushing will be timed for 2 minutes, and while being observed by a study team member during the duration of participation in the study. The plaque indices will be compared on the study subject to determine which sequence is more effective in removing dental plaque biofilm. Participants participation in this research will last no more than one (1) hour of one day.

**WHAT ARE THE KEY REASONS YOU MIGHT CHOOSE TO VOLUNTEER FOR THIS STUDY?**

There may or may not be any direct benefit for you to participate in this study, but you may learn information on maintaining oral health. For a complete description of benefits, refer to the Detailed Consent.

**WHAT ARE THE KEY REASONS YOU MIGHT NOT CHOOSE TO VOLUNTEER FOR THIS STUDY?**

Participation in this study is completely voluntary and you may choose not to participate or disenroll at any time. For a complete description of the risks, refer to the Detailed Consent.

**DO YOU HAVE TO TAKE PART IN THE STUDY?**

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any services, benefits, or rights you would normally have if you chose not to volunteer.

As a student, if you decide not to take part in this study, your choice will have no effect on your academic status or class grade(s).

As an employee, if you decide not to take part in this study, your choice will have no effect on your employment status.

### **WHAT IF YOU HAVE QUESTIONS, SUGGESTIONS OR CONCERNS?**

The Principal Investigator of this study is Justine Ponce, RDH, MS an Assistant Professor at the University of New Mexico Health Sciences Center, Department of Dental Hygiene. If you have questions, suggestions, or concerns regarding this study or you want to withdraw from the study, her contact information is 505-272-6688 or by email at [JStambaugh@salud.unm.edu](mailto:JStambaugh@salud.unm.edu).

If you have any questions or concerns about your rights as a volunteer in this research, contact staff in the University of New Mexico Health Sciences (UNMHSC) Human Research Review Committee (HRRC) between the business hours of 8AM and 5PM, Mountain Standard Time (MST), Monday-Friday at 505-272-1129.



## **DETAILED CONSENT**

### **ARE THERE REASONS WHY YOU WOULD NOT QUALIFY FOR THIS STUDY?**

Eligible participants must be at least 18 years of age, give consent, and follow instructions in English. Therefore, those who are not eligible will be exempt from this study. Prisoners are excluded from this study. Prospective participants are not being screened for pregnancy. The study sample will include adults who are able to perform the tasks of toothbrushing and flossing without aid. An intraoral examination screening for eligibility will be performed to ensure participants have teeth with contacts associated with the Ramfjord's Tooth Assignment of tooth Nos 3, 9, 12, 19, 25, 28. Qualified individuals are part of the Dental Medicine Department.

### **WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?**

The research procedures will be conducted at Novitski Hall, an established dental hygiene educational setting on UNM HSC Campus. You will need to come one time during the study for no more than one (1) hour.

### **WHAT WILL YOU BE ASKED TO DO?**

Not brushing or flossing for at least 12 hours prior to your study visit.

Chew on a dental plaque disclosing tablet and perform the tasks of toothbrushing and flossing without aid.

### **WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?**

There is little to no risk or discomfort to participants. While every effort will be made to protect participant's privacy, possible risk includes loss of privacy and confidentiality. All procedures will be thoroughly explained to participants and prior to any procedures are performed to assure patient comfort. To minimize the risk, collected data will be associated with the participant ID and stored securely.

You will not get any personal benefit from taking part in this study.

### **WHAT WILL IT COST YOU TO PARTICIPATE?**

There are no costs associated with taking part in the study.

### ***WHO WILL SEE THE INFORMATION THAT YOU GIVE?***

No personal or health information is required for the study, nor will it be collected to enroll in this study. A study team member will obtain the Informed Consent and any data collected will be associated with the participant ID.

### **CAN YOU CHOOSE TO WITHDRAW FROM THE STUDY EARLY?**

You can choose to leave the study at any time. You will not be treated differently if you decide to stop taking part in the study.

If you choose to leave the study early, data collected until that point will be discarded.

The investigators conducting the study may need to remove you from the study. This may occur for several reasons. You may be removed from the study if you are not able to follow the directions, they find that your participation in the study is more risk than benefit to you, or if you are not eligible.

### **WILL I BE PAID FOR PARTICIPATING IN THIS STUDY?**

You will not receive any rewards or payment for taking part in the study.

### **WHAT IF NEW INFORMATION IS LEARNED DURING THE STUDY THAT MIGHT AFFECT YOUR DECISION TO PARTICIPATE?**

You will be informed if the investigators learn new information that could change your mind about staying in the study. You may be asked to sign a new informed consent form if the information is provided to you after you have joined the study.

### **WILL YOU BE GIVEN INDIVIDUAL RESULTS FROM THE RESEARCH TESTS?**

You can be given feedback about the results from the study for purposes of this research, but no individualized results could be shared because there will be no individualized or personal information garnered.

### **WHAT ELSE DO YOU NEED TO KNOW?**

If you have not already received a copy of the Privacy Notice, you may request one. If you have any questions about your privacy rights, you should contact the University of New Mexico Health Sciences Privacy Officer between the business hours of 8am and 5pm Mountain Pacific Time, Monday-Friday at (505) 272-1493.

## INFORMED CONSENT SIGNATURE PAGE

This consent includes the following:

- Key Information Page
- Detailed Consent

You will receive a copy of this consent form after it has been signed.

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**Signature of research subject**

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**Date**

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**Printed name of research subject**

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Printed name of [authorized] person obtaining  
informed consent

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Date

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Signature of [authorized] person obtaining  
informed consent

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## Appendix C: Recruitment Email

Dear Department of Dental Medicine,

Professor Justine Ponce in collaboration with her research team is conducting research at the University of New Mexico to determine if toothbrushing and flossing sequence affect dental plaque biofilm removal. You are receiving this email because you are a part of Dental Medicine. The aim of this study is to determine if toothbrushing then flossing or flossing then toothbrushing are equally effective in plaque biofilm removal.

If you agree to participate in this study, an individual's participation will involve:

- Not brushing or flossing for at least 12 hours prior to your study visit
- Chewing a disclosing tablet, toothbrushing, and flossing without aid
- Will require no more than 1 hour of your time the day of

An email will be sent to the Department of Dental Medicine a week before and the day before to remind you to not brush or floss your teeth for at least 12 hours before the time of the study if you are participating in this study.

Upon enrollment in the study, a primary plaque index will be determined by a dental hygiene provider using a disclosing agent on your teeth. Secondly, participants will use method (A)

of toothbrushing for 2-minutes followed by flossing on the right side of the mouth and method (B) flossing then toothbrushing for 2-minutes on the left side of the mouth. Another plaque index will be performed by the dental hygiene provider on the participants teeth to determine if both methods were equally effective in plaque removal.

You do not have to participate in this study and your decision to participate is voluntary. If you would like to participate in this study, the consent form is attached in this email for you to read ahead of time and written consent will be taken the day of to complete and verify eligibility.

If you have any questions, please contact:

Principal Investigator:

Justine Ponce RDH, MS Assistant Professor  
University of New Mexico  
Division of Dental Hygiene  
Business (505) 272-6688  
JStambaugh@salud.unm.edu

## Appendix D: HRP-226 DSRF

## Departmental Scientific Review Form

The HRPO expects that a scientific review be conducted at the department level by either a Chair or designee with appropriate expertise in the given study area.



Human Research Protections Program  
University of New Mexico Health Sciences Center  
Phone: (505) 272 1129 Fax: (505) 272 0803  
[hppo@salud.unm.edu](mailto:hppo@salud.unm.edu)

### Study & Contact Information

Study Title: Toothbrushing and Flossing Sequence: The most effective way to reduce dental plaque biofilm

Principal Investigator: Justine Ponce

Name/Email JStambaugh@salud.unm.edu

Coordinator:

Name/Email

### Department Review Checklist

#### 1. \* Verify that the following criteria are met:

- ☒ The rationale for the study is clearly stated and the rationale is scientifically sound.
- ☒ The specific aims and objectives of this study are clearly stated and measurable.
- ☒ The standards for conducting this research are consistent with any guidelines of relevant professional associations and scholarly disciplines.
- ☒ The research uses procedures that are scientifically sound and appropriate to the purpose of the study with the least amount of risk.
- ☒ The study design is adequate to achieve the specific objectives of this study and the proposed participant population is appropriate.
- ☒ The data to be collected are necessary to meet the objectives of the study.
- ☒ Adequate literature review has been done to support and justify this study.
- ☒ Statistical considerations, including sample size and justification, estimated accrual and duration, and statistical analysis are clearly described and are adequate to meet the study objectives.
- ☒ The principal investigator & any other investigator involved in this research have sufficient resources/facilities to carry out the research.
- ☒ The principal investigator & any other investigator involved in this research are qualified by training and experience to personally conduct and/or supervise the research described in the protocol.
- ☒ The principal investigator & any other investigator involved in this research have completed all institutional credentialing requirements, if any, to conduct the research.
- ☒ Should the principal investigator depart from the institution prior to resolving the proper archival of study records, I will assume this responsibility and ensure that the confidentiality of study participants and their data is protected.

Provide an explanation for each box left unchecked (enter N/A if no boxes were left unchecked):

#### 2. \* Reviewer Determination:

- ☒ Approved
- ☐ Disapproved

#### 3. Notes:

I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and accept the obligation to assure compliance with all applicable federal regulations and state laws, institutional policies and procedures, and the requirements and determinations of the UNM Human Research Protections Office (HRPO) with respect to this research.

By signing, I hereby approve this project, based on my review:

<u>Arthur Gordon</u>	<u>Dental Medicine</u>
Name of Department Chair or Designee & Title	Department
<u>[Signature]</u>	<u>3/13/2023</u>
Signature of Department Chair or Designee	Date

# Appendix E: Data Collection Sheet

## PCR Data Collection Sheet

Study 23-167: Toothbrushing and Flossing Sequence for Biofilm Control

PT	Participant #ID
PCR Equation	Total Surfaces with Biofilm ÷ 12 (# of teeth x 4)

PT ID\_\_\_\_\_

PCR- PRE OHI

PCR-POST OHI

	Tooth #'s:						
Right Side (Method A)	3	25	28		3	25	28
	M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____		M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____
Left Side (Method B)	9	12	19		9	12	19
	M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____		M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____	M._____ D._____ L._____ F._____

PRE Score Right\_\_\_\_\_

POST Score Right\_\_\_\_\_

PRE Score|Left\_\_\_\_\_

POST Score Left\_\_\_\_\_



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