The Effects of Flossing with a Chlorhexidine Solution on Interproximal Gingivitis: A Randomized Controlled Trial

by

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ABSTRACT

Background: Gingivitis, which is an inflammatory response of the gingival tissues to bacterial plaque, can be treated by brushing and flossing or using chlorhexidine (CHX).

Objective: To determine whether floss presoaked in CHX would improve oral health relative to floss presoaked in placebo solution.

Materials and methods: A 3 month, double-blinded, parallel, randomized control trial was conducted on 27 adults with a minimum of 10 bleeding sites, who were randomly assigned to dental floss with 0.12% CHX or a placebo solution (QS). Scaling, rubber cup prophylaxis, and flossing instructions were performed at Week -1. Subjects were assessed for probing depth (PD) with bleeding on probing (BOP), gingival index (GI), plaque index (PI), and stain index (SI) at Weeks 0, 6, and 12. Flossing compliance was monitored by self-reports and yards of dental floss used. Student t-tests, ANCOVA and Wilcoxon Signed-Rank were conducted.

Results: Self-reported median flossing compliance was 100% for CHX and 93% for QS. A statistically significant reduction for PD in the CHX group at Week 6 (p = 0.029) and specifically, for the gingivitis sites at Week 6 (p = 0.006) and Week 12 (p = 0.005). Reductions in BOP were statistically significant for CHX group with moderate gingivitis (p = 0.0078). The CHX group had a statistically significant reduction in BOP in all areas of the oral cavity with the largest reductions occurring in the anterior areas (p = 0.011).
All GI scores were statistically significant from Weeks 0 to 12 (p < 0.0001). Although not statistically significant, PI appeared to be constant for CHX group and increasing for QS group. The two groups did not differ significantly for SI.

Conclusion: CHX applied via dental floss significantly reduced probing depths in shallow sulci and bleeding in subjects with moderate gingivitis. Further studies are suggested to determine the effects of CHX with different interdental aids, higher concentrations of CHX, and subjects with moderate to severe gingivitis.

Funding: CFDHRE and BCDHA

Key words: gingivitis, chlorhexidine, dental floss, RCT, bleeding on probing, gingival and plaque indices.
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DEDICATION

To my children, Kaitlin and Jordan
CO-AUTHORSHIP STATEMENT

The randomized controlled trial (RCT) was co-authored by Dr. Donald M. Brunette and Dr. Edward E. Putnins. I designed the study as a double-blinded, parallel RCT with the suggestion from Dr. Putnins to have a 3 month study as opposed to a 6 month study and to do the baseline data collection approximately one week after the subjects' debridement visit. Otherwise the study followed the research protocol that I designed and Dr. Brunette approved. I submitted and received approval for the study, which included study amendments, from the University of British Columbia's Clinical Research Ethics Board. I applied for a competitive grant and received funding for the study from the Canadian Foundation for Dental Hygiene Research and Education. Since I do not have a faculty position, Dr. Brunette had authority over the funding account, but I managed the expenses. I recruited all the study subjects and collected all the clinical data. I arranged an agreement with a private practice dentist to use his dental clinic for the clinical aspects of the RCT. I did the data entry and conducted all the initial statistical analyses. Mr. Ryan Woods, statistician, verified my findings. I prepared the preliminary manuscript and received some feedback from Dr. Putnins and Dr. Brunette, which I subsequently incorporated into the final manuscript. I will present the findings of the RCT at the International Dental Hygiene Conference in Toronto in July 2007.
1 Introduction

1.1 Thesis Overview

Gingivitis, which is an inflammatory response of the gingival tissues to dental plaque,\(^1\) can be treated by mechanical \(^{1-4}\) or chemical methods.\(^5\) Mechanical methods such as, toothbrushing and dental flossing, treat gingivitis by removing dental plaque.\(^{1-4}\) Chemical methods such as, rinsing with chlorhexidine (CHX), treat gingivitis by inhibiting dental plaque.\(^5\) This thesis explores the novel application of CHX with dental floss such that, the chemical properties of CHX and the mechanical properties of dental floss are combined to treat gingivitis.

1.1.2 Thesis Organization

The thesis introduction provides information about gingivitis and the current methods for treating gingivitis. The rationale for the thesis and thesis objectives are found at the end of the introduction. Chapter 2 is a slightly modified version of a literature review published in the Canadian Journal of Dental Hygiene of CHX and the various methods of applying it in the oral cavity.\(^5\) Since there are numerous types of dental floss available on the Canadian market, an in-vitro study, which can be found in Appendix A.1, was conducted to determine the best type of floss to use in the randomized controlled trial (RCT). The in-vitro study demonstrated that the Johnson and Johnson® waxed dental floss (Reach, McNeil-PPC Inc., Montreal, Quebec) was the best floss for carrying the CHX solution. Chapter 3 describes the RCT that compared dental floss presoaked in CHX solution to a dental floss presoaked in a placebo solution on the clinical signs of
interproximal gingivitis. The RCT demonstrated that the dental floss with the CHX had a statistically significant effect on various periodontal indices compared to the dental floss with placebo solution. The concluding chapter contains the overall thesis conclusion with suggestions for future studies.

1.2 Gingivitis

1.2.1 Gingivitis Etiology

Gingivitis is an endemic oral disease that affects children, adolescents, and adults. Although there are different types of gingival diseases, dental plaque-induced gingivitis is the most prevalent and is the focus of this thesis. Dental plaque, which is an oral biofilm, is a complex community of synergistic, pathogenic bacteria. Loe, Theilade, and Jensen (1965) investigated the cause and effect relationship between dental plaque and gingivitis and demonstrated that the presence of dental plaque initiated and exacerbated gingival diseases, whereas the removal of dental plaque resulted in gingival health.

1.2.2 Gingivitis Stages

Gingivitis may progress through a series of stages labeled as initial, early, and established lesions. In all stages, the signs and symptoms of gingivitis are restricted to the gingivae.

1.2.2.1 Initial Gingivitis Lesion

The initial stage of gingivitis is difficult to clinically differentiate from healthy gingival tissues. Although the initial lesion may appear healthy there are differences in the micro
flora. The micro flora of healthy tissues consists predominately of gram positive
Streptococci, facultative species of Actinomyces, and Veillonella species, with few motile
forms.\textsuperscript{7,10} As gingivitis develops, the micro flora changes to a complex gram negative
and spiral flora consisting of increasing numbers of Actinomyces naeslundii, Actinomyces
viscosus, Fusobacterium nucleatum, Tannerella spp. (formerly Bacteriodes spp.), and
Porphyromonas gingivalis.\textsuperscript{7,10}

Histological studies also demonstrate differences between a healthy site and an initial
gingivitis lesion. Initial lesions demonstrate the presence of acute inflammation such as,
increased gingival cervical fluid flow, transmigrated neutrophils, deposited fibrin, and
destroyed connective tissue collagen.\textsuperscript{7} The inflammatory infiltrate in initial lesions is
restricted to 5-10\% of the marginal gingival connective tissue.\textsuperscript{7}

1.2.2.2 Early Gingivitis Lesion

In the early lesion stage, gingivitis becomes clinically visible. The gingival tissues will
appear red, edematous, shiny, and smooth, and will bleed easily with slight stimulation.\textsuperscript{11}
Gingival bleeding is one of the earliest clinical signs of inflammation.\textsuperscript{11,12} Histological
studies indicate moderate to intense mononuclear inflammatory infiltrate with numerous
swollen blood vessels and inter-tissue hemorrhaged points that bleed upon slight
provocation within the connective tissues of an early gingivitis lesion.\textsuperscript{7,13} Lymphocytes
dominate the inflammatory population and collagen damage extends to 5 -15\% of the
marginal gingival connective tissue.\textsuperscript{7}
1.2.2.3 Established Gingivitis Lesion

The established lesion, which is commonly referred to as chronic gingivitis, may clinically manifest itself as an early lesion or it may appear fibrotic and cyanotic as the blood becomes stagnant in the area over time. Histological studies of the established lesion are characterized by predominately plasma cells and B-lymphocytes, neutrophils in the junctional and pocket epithelium, and macrophages in the lamina propria.

Although gingivitis may progress through all 3 stages, it may also be stopped or reversed at any stage with meticulous plaque control.

1.2.3 Gingivitis Treatment

1.2.3.1 Mechanical treatment

One method for treating gingivitis is to mechanically remove the dental plaque. Studies have shown that toothbrushing and dental flossing will significantly reduce the bleeding and inflammation that is associated with gingivitis. Although toothbrushing removes dental plaque from accessible tooth surfaces it cannot de-plaque the interproximal surfaces. Since gingivitis is more prevalent interproximally than any other area of the oral cavity, the daily use of dental floss is particularly important for oral self-care.
1.2.3.2 Chemical treatment

Another method for treating gingivitis is to use an anti-microbial mouth rinse. Anti-microbial mouth rinses treat gingivitis by inhibiting the formation of dental plaque or controlling the deleterious bacterial by-products.\textsuperscript{24} Although numerous studies have been conducted on various chemical agents for controlling dental plaque, CHX has been referred to as the gold standard because of its substantivity.\textsuperscript{24} A gold standard is a positive control against which the effectiveness of other alternative agents is compared.\textsuperscript{24} Substantivity is the ability of a drug or agent to be absorbed and then released in active form hours after the initial dose.\textsuperscript{24} CHX possesses substantivity because it can be found in an active form in saliva and on oral surfaces 5 and 12 hours, respectively, later.\textsuperscript{24}

1.2.3.2.1 Chlorhexidine

Numerous studies have demonstrated the anti-plaque and anti-gingivitis properties of CHX.\textsuperscript{5} (See Chapter 2). However, patient compliance is low because of the dark brown stain that develops on the teeth within a few days of use.\textsuperscript{25-29} Three out of four people develop the tooth stain.\textsuperscript{25-29} Therefore, numerous studies have investigated methods of applying CHX such that the anti-plaque and anti-gingivitis properties are retained without the tooth stain.\textsuperscript{5} (See Chapter 2).
1.3 Thesis Rationale

Of particular relevance to this thesis was Kinane et al.'s study (1992) in which a floss holder device and CHX was used to reduce bleeding gingival tissues. Kinane et al. attempted to combine the mechanical effectiveness of dental floss with the chemical effectiveness of CHX to determine whether the combined approach would be more effective than a flossing device with placebo solution or conventional dental floss with no solutions. In this short-term RCT, subjects were asked to floss their teeth with the specially designed floss holder, which sprayed CHX onto the floss after it was placed into the interproximal area. The study demonstrated a statistically significant difference between the floss holders and conventional floss (p = 0.024), but did not demonstrate a statistically significant difference between the CHX and placebo floss holders. Although the researchers suggested that the dosage of CHX was too low, another explanation is that the CHX did not penetrate the interproximal area because it was blocked by the dental floss.

In this thesis, the dental floss was immersed in the CHX solution with the intention of having the dental floss carry the CHX into the interproximal area. In Chapter 3, the RCT demonstrated that the dental floss with the CHX solution was superior to the dental floss with the placebo solution for reducing probing depths and bleeding on probing and was able to do so without tooth staining.
1.4 Thesis Objectives

1) To determine which dental floss soaked in a 0.12% CHX solution would exert a bacteriostatic effect in-vitro.

2) To determine whether dental floss soaked in 0.12% CHX solution would improve various clinical periodontal indices.

3) To determine if the application of CHX with dental floss would result in tooth staining.
1.5 References


2 A REVIEW OF THE DIFFERENT METHODS OF APPLYING CHLORHEXIDINE IN THE ORAL CAVITY *

2.1 Chlorhexidine Background Information

2.1.1 History and use

Chlorhexidine (1.6-bis-4-chloro-phenyl-diguanido-hexane) (CHX) is a synthetic cationic detergent.\(^1\) It was marketed in 1953 as the antiseptic cream, Hibitane\(^{TM}\), but in 1970 was found to be effective against dental plaque.\(^2\) Dental plaque is the etiological cause of plaque-induced gingivitis.\(^3\) In a short-term, experimental gingivitis model study, Løe and Schiøtt (1970) demonstrated that rinsing with 0.2% CHX twice a day completely prevented plaque formation without any mechanical interventions.\(^2\) Numerous studies have demonstrated CHX's ability to inhibit de novo plaque formation and to reduce the bleeding and inflammation associated with gingivitis.\(^2,4-51\) In Canada, CHX rinse is available by prescription for the treatment of gingivitis.

2.1.2 Effects on oral bacteria

CHX is effective against gram-positive and gram-negative bacteria, yeasts, dermatophytes, and some lipophilic viruses.\(^1,52\) In high doses, CHX can kill bacteria by damaging the cytoplasmic membrane, such that the osmotic equilibrium is no longer

* A version of this chapter has been published. Imai PH. A review of the different methods of applying chlorhexidine in the oral cavity. CJDH. 2006; 40: 69-79.
maintained and the cytoplasm becomes coagulated with the formation of phosphated complexes. In low doses, CHX cannot kill bacteria, but it can inhibit the bacteria’s ability to adhere to tooth surfaces by binding the carboxyl and phosphate groups of the dental pellicle.

## 2.1.3 Safety

CHX demonstrated no adverse effects for reproduction, skin sensitizations, and eye irritations in animal models. CHX has very low toxicity in humans and animals because it is poorly absorbed by the gastrointestinal tract. Reported cases of CHX anaphylaxis are rare. Krautheim (2004) reported one subject who had an anaphylactic reaction to CHX, but it was thought that the reaction occurred because CHX was introduced into the bloodstream through broken skin. The sensitization rate of CHX is less than 2%, with prolonged and repeated contact required to develop contact sensitivity. Long-term, epidemiological studies have demonstrated CHX’s safety, continual effectiveness, and lack of resistant bacterial strain development.

## 2.1.4 Characteristics

### 2.1.4.1 Substantivity and gold standard

CHX possesses substantivity, which is the ability of a drug to be adsorbed and later released in active form long after the initial dose. For example, CHX is released by the oral tissues in active form 8-12 hours after the initial dose. Since substantivity enables CHX to have continuous anti-microbial effects even with less frequent applications, CHX is considered the gold standard for anti-microbial mouth rinses. A gold standard is a
positive control against which alternative agents or tests are compared.\textsuperscript{52}

2.1.4.2 Interactions

Since CHX is strongly cationic, it may be rendered inactive in the presence of anionic detergents such as sodium lauryl sulfate, which is found in toothpastes.\textsuperscript{6} Although researchers and clinicians recommend a minimum waiting time of 30 minutes between the use of toothpastes and CHX, Van Strydonck \textit{et al.} (2004) found there was no inactivation of the CHX as long as the person rinsed his or her mouth with water after brushing with toothpaste.\textsuperscript{56}

2.1.4.3 Side effects

Tooth stain is a common side effect of CHX. Three out of four subjects will develop a brown stain on the teeth, tongue, and composite and porcelain restorations within a few days of using CHX.\textsuperscript{1, 2, 7, 57-59} The intensity and coverage of the tooth stain increases with the intake of tea, red wine, coffee, and tobacco possibly because the denaturing pellicle proteins allow the formation of iron or tin sulfides to form pigmented products on the teeth.\textsuperscript{59, 60}

Other side effects of CHX are bitter, metallic taste (12\%), changes in taste sensation (88\%), and occasional epithelial desquamation (6\%).\textsuperscript{57, 61, 62} Desquamations are usually observed in subjects using CHX concentrations of 0.2\% or more.\textsuperscript{1, 8, 9, 52}
2.1.5 Modes of application

CHX has been applied by various methods. The most common method is the mouth rinse, followed by gels, sprays, and other novel methods. The purpose of these different application methods is to find a method that would enhance the anti-plaque and anti-gingivitis properties of CHX, while simultaneously minimizing its side effects.

2.2 Chlorhexidine Mouth Rinses

2.2.1 Original concentration: rationale for use and optimal dose

CHX has been traditionally dispensed as a 0.2% mouth rinse. Løe and Schiøtt (1970) chose 0.2% in the initial CHX clinical trial because this concentration was used to irrigate the eyes and therefore, was felt to be safe. Long considered the gold standard, 0.2% CHX is effective at inhibiting new plaque formation and controlling the clinical signs of gingivitis. In experimental gingivitis studies, 0.2% CHX returned subjects to gingival health without mechanical interventions. CHX also resolved experimental gingivitis better than manual tooth brushing (p < 0.05). The optimal dose for 0.2% CHX mouth rinse is 20 ml per day.

Although the mouth rinse is easy to use and accepted by subjects, long-term compliance has been poor because of the extrinsic brown stain that forms on the teeth and oral tissue within a few days of use. Therefore, studies have explored lower concentrations, different formulations, and different methods of applying CHX to address the staining
issue, whilst maintaining its anti-plaque and anti-gingivitis properties.

2.2.2 Lower concentrations

2.2.2.1 Retention and Optimal dose

Lower concentrations of CHX were studied to determine if the beneficial properties of CHX could be maintained whilst eliminating tooth staining. Although 0.2 % CHX was known to have substantivity,\textsuperscript{52} it was unknown whether a lower concentration would also possess substantivity.

Bonesvoll, Lökken and Rølla (1974) demonstrated that CHX retention was proportional to its concentration such that, the higher the concentration, the more that was retained.\textsuperscript{55} For example, 1.8 mg (+ 0.4) of CHX was retained in the oral cavity at a 0.05% concentration, but 10.5 mg (+ 3.4) was retained at the 0.4% concentration.\textsuperscript{55} Sreenivasan et al. (2004) found a significant dose-dependent effect such that higher CHX concentrations had significantly stronger effects on oral bacteria.\textsuperscript{34}

The optimal dose for 0.12% CHX is 30 ml per day.\textsuperscript{52}

2.2.2.2 Short-term studies

2.2.2.2.1 Compared to other agents

In short-term studies, the anti-plaque and anti-gingivitis efficacy of 0.12% CHX has been compared to other agents or placebo. Ramberg et al. (1996) used an experimental gingivitis model to compare 0.12% CHX, triclosan, and placebo, and demonstrated a statistically significant lower plaque score, but not gingival inflammation and gingival
crevicular fluid scores, for CHX. Horwitz et al. (2000) compared 0.12% CHX to a solution of amine and stannous fluoride in post-surgical subjects and found no differences. The lack of difference between the CHX and fluoride solution may have been attributed to the recent periodontal surgery and the subjects' enhanced post-surgical oral hygiene. Eaton et al. (1997) demonstrated a statistically significant reduction in plaque and bleeding in a multi-centre, general dental practice study with subjects using a 0.12% CHX rinse even though these subjects already had low baseline plaque and gingival scores.

2.2.2.2 Compared to mechanical plaque removal

Comparisons between mechanical plaque removal and lower concentrations of CHX have also been reported. Caton et al. (1993) compared the adjunctive use of 0.12% CHX and mechanical cleaning to mechanical cleaning alone in the treatment of inter-dental gingivitis. The subjects performing only inter-dental cleaning and tooth brushing had a statistically significant reduction in bleeding compared to the group tooth brushing and rinsing with CHX. Although the results support previous findings that tooth brushing alone is ineffective in cleaning the inter-dental area, it also demonstrates the inability of CHX mouth rinses to penetrate the inter-dental area, an area where gingivitis is prevalent.

In short-term studies, the efficacy of CHX on plaque formation and gingivitis appears to be only slightly better or equivalent to other test solutions, placebos or mechanical cleaning. Possible explanations for the small differences between CHX and other agents are the Hawthorne effect and the lingering beneficial effects from the initial professional
debridement. The Hawthorne effect refers to a phenomenon in which people, who are being observed in a study or received new or increased attention, temporarily change their behavior or performance. In gingivitis efficacy studies, the Hawthorne effect manifests itself as increased motivation to comply with the study protocol. For example, subjects who usually do not dental floss will floss daily while in a study.

### 2.2.2.3 Long-term studies

According to Overholser (1988), studies of six months or longer have the following advantages over short-term studies:

1. Six months simulates a common hygiene maintenance interval in private practice.
2. Subjects will likely begin and end the study with a professional debridement. Having all the subjects begin with a plaque score near or at zero facilitates easier comparisons between the treatment groups later.
3. The effects of the initial scaling and root planing will have mitigated by six months.
4. The development of toxic and other adverse effects are more likely to become known.
5. Qualitative and quantitative changes in the subject's oral flora can be monitored, especially for the emergence of gram negative, anaerobic or motile bacteria. Six months also allows researchers to determine if the treatment produces resistant forms of bacteria.
6. The Hawthorne effect will gradually lessen over time.
In long-term studies, 0.12% CHX rinses have been found to be effective in reducing plaque and gingivitis compared to placebo.\textsuperscript{20, 24} Grossman \textit{et al.} (1986) demonstrated a 37% reduction in gingivitis occurrence, 39% reduction in gingival severity, 44% reduction in gingival bleeding, and 61% reduction in plaque scores for the CHX group compared to the placebo group.\textsuperscript{24} The increasing gingival severity in the placebo group over time indicated the diminishing beneficial effect from the initial professional prophylaxis.\textsuperscript{24}

\subsection*{2.2.2.3.1 Compared to other agents}

Charles \textit{et al.} (2004) compared 0.12\% CHX to an essential oil rinse and a placebo rinse.\textsuperscript{20} The CHX had statistically significant reductions in gingival (p < 0.001) and plaque (p < 0.001) scores compared to placebo.\textsuperscript{20} When compared to an essential oil mouth rinse (Listerine\textsuperscript{®} antiseptic, Pfizer Inc., Morris Plains, NJ, USA), CHX did not exhibit any statistically significant differences for plaque and gingival scores.\textsuperscript{20} Subjects in the CHX group had a statistically significant increase in calculus (0.45) and stain (2.08) compared to the essential oil rinse (0.24 and 0.33, respectively) and placebo (0.21 and 0.01, respectively).\textsuperscript{20}

Hoffman \textit{et al.} (2001) compared 0.06\% CHX mouth rinse to a solution of 0.06\% CHX with 250 parts per million sodium fluoride.\textsuperscript{27} The study included a positive control, 0.1\% CHX, and two negative controls, water and a fluoride solution containing 250 parts per million amine fluoride and stannous fluoride.\textsuperscript{27} The results of this study can be found in Table 2.1. Hoffmann \textit{et al.} (2001) speculate that the Hawthorne effect may have been
present because the 3 month results were better than the six month results, but it is also possible that the subgingival micro flora had not re-established itself until after 3 months.

Table 2.1 Comparing the effects of 0.06% chlorhexidine with sodium fluoride and 0.06% chlorhexidine to a positive control of 0.10% chlorhexidine and negative controls of water and amine fluoride with stannous fluoride, on gingival health using plaque, gingival, and discolouration indices at three and six months in Hoffmann et al. (2001)27

<table>
<thead>
<tr>
<th></th>
<th>PII (median scores)</th>
<th>GI (median scores)</th>
<th>DI (median scores)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 M</td>
<td>6 M</td>
<td>3 M</td>
</tr>
<tr>
<td><strong>Month</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (Negative control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.55</td>
<td>0.72</td>
<td>0.28</td>
</tr>
<tr>
<td>Amine fluoride with stannous fluoride (Negative control)</td>
<td>0.23</td>
<td>0.29</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>*P = 0.0456</td>
<td>*P = 0.0150</td>
<td>*P = 0.287</td>
</tr>
<tr>
<td>0.06% CHX + Sodium fluoride</td>
<td>0.20</td>
<td>0.27</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>*P = 0.0022</td>
<td>*P = 0.0130</td>
<td>*P = 0.151</td>
</tr>
<tr>
<td>0.06% CHX (Positive control)</td>
<td>0.14</td>
<td>0.25</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>*P = 0.0007</td>
<td>*P = 0.0077</td>
<td>*P = 0.183</td>
</tr>
<tr>
<td>0.10% CHX (Positive control)</td>
<td>0.15</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>*P = 0.0013</td>
<td>*P = 0.0007</td>
<td>*P = 0.045</td>
</tr>
</tbody>
</table>

PII = Plaque index, GI = Gingival index, DI = Discolouration index
CHX = Chlorhexidine
* = Significant findings, p < 0.05
NSF = No significant findings
2.2.2.3.2 Compared to mechanical plaque removal

Flemmig et al. (1990) compared 0.06% CHX via mouth rinsing and oral irrigation to water irrigation and toothbrushing and found that CHX, regardless of application and the water irrigation group all had statistically significant reductions for gingival index and bleeding on probing compared to the toothbrushing group ($p \leq 0.05$). Details of Flemmig et al.'s study is in Table 2.2.

Table 2.2 Comparing the effects of 0.06% chlorhexidine administered by oral irrigator or mouth rinse to water irrigation and toothbrushing on gingival health using gingival index, bleeding on probing, plaque, calculus, and stain indices, and pocket probing depths after six months of treatment.

<table>
<thead>
<tr>
<th></th>
<th>Gingival index</th>
<th>Bleeding On Probing</th>
<th>Plaque index</th>
<th>Calculus index</th>
<th>Staining index</th>
<th>Pocket probing depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06% CHX oral irrigator</td>
<td>↓ 42.5%</td>
<td>↓ 35.4%</td>
<td>↓ 53.2%</td>
<td>↑ 276.4%</td>
<td>↑ 68.9%</td>
<td>↓ 4.6%</td>
</tr>
<tr>
<td>0.06% CHX mouthrinse</td>
<td>↓ 24.1%</td>
<td>↓ 15.0%</td>
<td>↓ 43.3%</td>
<td>↑ 273.2%</td>
<td>↑ 74.2%</td>
<td>NSF</td>
</tr>
<tr>
<td>Water irrigator</td>
<td>↓ 23.1%</td>
<td>↓ 24.0%</td>
<td>NSF</td>
<td>↑ 7.1%</td>
<td>NSF</td>
<td>NSF</td>
</tr>
</tbody>
</table>

CHX = Chlorhexidine  
$\downarrow$ = Percentage reduction in scores compared to toothbrushing control  
$\uparrow$ = Percentage increase in scores compared to toothbrushing control  
NSF = No significant findings between treatment group and toothbrushing control

The beneficial effects of water irrigation were possibly due to a reduction in bacteria or toxic bacterial by-products, but this is unknown since microbiological tests were not conducted. Mechanical stimulation of the gingival tissues may also explain the positive results of water irrigation.
2.2.2.4 Modes of application

2.2.2.4.1 Oral irrigation

Cumming and Loe (1973) tested the plaque efficacy of various lower concentrations of CHX as mouth rinses and via an oral irrigator.\textsuperscript{21} Concentrations as low as 0.075% were effective as a mouth rinse as long as the total volume of solution was increased.\textsuperscript{21} For example, 100 ml of 0.075% CHX was equivalent to 20 ml of 0.2% CHX for plaque inhibition.\textsuperscript{21} Irrigating with 700 ml of 0.05% CHX was also effective.\textsuperscript{21} However, since the volumes were so large, subject compliance and acceptance was low.\textsuperscript{21} Therefore, rinsing with 50 ml of 0.075% to 0.1% CHX or irrigating with 400 ml of 0.025% to 0.05% CHX was suggested for inhibiting plaque formation.\textsuperscript{21} The lower concentrations had less tooth staining and minimal bitter taste.\textsuperscript{21}

2.2.2.4.2 Toothbrush and swabs

CHX has also been applied with toothbrushes or foam swabs.\textsuperscript{43, 46, 48} Applying 0.10% and 0.15% CHX solutions with toothbrushes were effective in reducing plaque scores (66% and 72%, respectively) compared to placebo.\textsuperscript{43} The 0.15% CHX reduced mean gingival index scores by 58% and the 0.10% concentration reduced scores by 57% compared to placebo.\textsuperscript{43} Stain intensity increased with increasing CHX concentrations.\textsuperscript{43} For example, 92% of the subjects using 0.15% CHX had tooth staining compared to 17% of the subjects using 0.05% CHX.\textsuperscript{43} Although brushing with CHX did not eliminate tooth staining, burning sensations and desquamative lesions were absent because the tooth brushing method may have prevented the CHX from contacting the mucosal membranes.\textsuperscript{43}
CHX solution has also been applied with a foam brush for subjects who were unable to use a conventional toothbrush.\textsuperscript{46, 48} Although the studies demonstrated an anti-plaque and anti-gingivitis effect, there were significant carry-over effects as the subjects were crossed over from one treatment to the next.\textsuperscript{46, 48}

\textbf{2.2.2.5 Different formulations}

\textbf{2.2.2.5.1 To reduce tooth staining}

Studies on different CHX formulations have also been conducted to minimize or eliminate the side effects of tooth staining and poor taste. Addy \textit{et al.} (1991) compared the efficacy of 0.12\% CHX and a reformulated 0.1\% CHX rinse (Pierre Fabre, Castres, France) and found that the anti-plaque and anti-gingivitis properties of the 0.1\% rinse were reduced compared to the 0.12\% rinse.\textsuperscript{17} However, the anti-plaque effect of the 0.1\% formulation was not clear because of significant cross-over effects. Although \textit{in vivo} and \textit{in vitro} pilot studies by Addy \textit{et al.} (1991) demonstrated a reduction in tooth staining at the expense of anti-plaque effects, the main study did not include a stain index therefore, comparisons between the 0.1\% and 0.12\% CHX rinses were incomplete.\textsuperscript{17}

Joyston-Bechal and Hernaman (1993) added 0.5\% sodium fluoride to 0.5\% CHX to determine if this formulation would result in less stain, while continuing to have an effect on plaque formation and gingivitis.\textsuperscript{28} After eight weeks, the CHX with fluoride group had a mean plaque score of $0.4 \pm 0.2$ ($p < 0.001$) and a mean bleeding score of $0.1 \pm 0.1$ ($p < 0.001$) compared to placebo $0.95 \pm 0.35$ and $0.45 \pm 0.30$ ($p < 0.05$), respectively.\textsuperscript{28}
Although the new formulation reduced plaque and bleeding scores, staining scores were less clear. The CHX with fluoride subjects had significantly higher baseline stain scores than the placebo group and this difference was not controlled in the statistical analysis; therefore, it is unclear if the test formulation was successful in having significantly less tooth staining.

2.2.2.5.2 To reduce bitter taste

To improve the taste of CHX mouth rinse, new formulations replaced the alcohol with 0.5% cetylpyridinium chloride (CPC).\textsuperscript{30,32}

Quirynen \textit{et al.} (2001) had subjects rate the taste and tooth staining of four CHX rinses: 0.2% CHX with alcohol, 0.12% CHX with alcohol, 0.12% CHX with 0.5% sodium fluoride, and 0.12% CHX with CPC.\textsuperscript{30} Although the 0.12% CHX rinses with CPC and alcohol were clinically and microbiologically effective in retarding new plaque formation, the subjects preferred the taste of the 0.12% CPC CHX rinse ($p < 0.01$).\textsuperscript{30} There were no significant differences among the CHX rinses for tooth staining.\textsuperscript{30}

Santos \textit{et al.} (2004) and Van Strydonck \textit{et al.} (2005) also demonstrated that 0.12% CPC CHX rinse is not significantly different than 0.12% CHX rinse with alcohol on plaque accumulation and bacterial counts and the only advantage of 0.12% CPC CHX was better taste.\textsuperscript{32,35}
2.2.3 Mouth rinse summary

Although 0.2% CHX mouth rinse is an effective anti-plaque and anti-gingivitis agent, side effects such as tooth staining, changes in taste perception, and poor taste of the solution have limited its long-term use. Subsequent studies of lower CHX concentrations have shown similar anti-plaque and anti-gingivitis effects. In particular, the 0.12% cetylpyridinium formulation was shown to taste better. Although applying these lower concentrations of CHX by oral irrigation or toothbrushing reduced epithelial desquamations, tooth staining remained a problem. Therefore, other methods of applying CHX were explored.

2.3 Chlorhexidine Gels

2.3.1 Rationale for use and optimal dose

The 1% CHX gel was developed to incorporate CHX into a subject's oral self-care by substituting the subject's toothpaste with the CHX gel. This application method would theoretically provide adjunctive benefits to mechanical oral hygiene for the treatment of gingivitis.

To determine if a CHX gel would be retained intraorally, Bonesvoll (1978) tested 1% CHX gel with various concentrations of CHX mouth rinses. Four milligrams of CHX was retained after toothbrushing with 1 gram of 1% CHX gel, which is similar to rinsing
with 10 ml 0.1% CHX mouth rinse for one minute. The length of brushing time had little influence on CHX gel retention, with times as short as 15 seconds having high retention levels. According to Gjermo, Bonesvoll, and Rölla (1974), the plaque inhibiting effect of CHX is related to the amount of CHX retained. The optimum dosage for CHX gel is 40 mg per day. 

2.3.2 Short-term studies

When CHX gel is applied twice a day, plaque and gingivitis scores are reduced significantly. Bassiouny and Grant (1975) reported statistically significant reductions in plaque and gingival index scores between a 1% CHX gel and placebo gel. Lie and Enersen (1986) also reported statistically significant reductions in plaque and bleeding sites in maintenance care subjects using 1% CHX gel. However, both studies reported significant tooth staining with the CHX gel.

2.3.3 Long-term study

Cutress et al. (1977) demonstrated a statistically significant difference for tooth staining, but not for plaque and gingival scores between 1% CHX gel and placebo gel over 6 months. The study population, which consisted of mentally challenged children, may have been too challenging to effectively apply the CHX gel and hence, no beneficial effects were demonstrated.
2.3.4 Modes of application

2.3.4.1 Applied by fingers

CHX gel may be applied with toothbrushes, fingers, toothpicks, or trays. Pai et al. (2004) compared the application of one gram 1% CHX gel via subject’s index finger to placebo gel, 0.2% CHX mouth rinse, and neem extract gel (Azadirachta indica, a plant found in India and southern Asia, which is commonly used for oral health care). The CHX gel, CHX rinse, and neem gel had statistically significant reductions in plaque and gingival scores compared to the placebo gel (p< 0.05). Details of the results are in Table 2.3. The results may be subject to bias however, because there was no examiner blinding.

Table 2.3 Comparison of 1% chlorhexidine gel and Neem extract gel to a positive control of 0.2% chlorhexidine mouth rinse and a negative control of placebo gel on mean plaque and gingival scores at six weeks in Pai et al. (2004)

<table>
<thead>
<tr>
<th></th>
<th>Mean plaque score with SD</th>
<th>Mean gingival score with SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control placebo gel</td>
<td>1.31 ± 0.20</td>
<td>1.140 ± 0.26</td>
</tr>
<tr>
<td>0.2% CHX mouth rinse</td>
<td>0.98 ± 0.20</td>
<td>0.92 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>*p&lt; 0.05</td>
<td>*p&lt; 0.05</td>
</tr>
<tr>
<td>1% CHX gel</td>
<td>0.62 ± 0.29</td>
<td>0.52 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>*p&lt; 0.05</td>
<td>*p&lt; 0.05</td>
</tr>
<tr>
<td>Neem extract gel</td>
<td>0.63 ± 0.24</td>
<td>0.60 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>*p&lt; 0.05</td>
<td>*p&lt; 0.05</td>
</tr>
</tbody>
</table>

CHX = Chlorhexidine
* = Significant result compared to placebo gel, p ≤ 0.05
2.3.4.2 Applied by toothpicks
Bastos Freitas et al. (1992) conducted a one-week study applying 1% CHX gel with toothpicks. There was no statistically significant difference in plaque scores between the CHX gel and placebo gels because of the mechanical cleaning effect of the toothpick and the CHX gel accumulating on the buccal surface instead of being carried into the interproximal area.

2.3.4.3 Applied by tray
Pannuti et al. (2003) used 0.5% CHX gel in trays twice a day and found statistically significant reductions (p < 0.001) for interdental bleeding compared to placebo gel, however this difference may have been attributed to the 6.1% increase in bleeding in the placebo group. The CHX group had a statistically significant increase in tooth staining compared to placebo. The total daily dose of CHX in this study was 120 mg, which is three times the optimal dose for CHX gel.

Francis et al. (1987) compared 1% CHX gel in trays to 0.2% CHX mouth rinse and 0.2% CHX spray in handicapped children. The CHX was applied twice a day, regardless of application method, for four weeks followed by a three week washout period between treatments. Although all three methods were effective in reducing plaque and gingival bleeding scores, the gel was significantly more effective. The better results with the CHX gel was attributed to better coverage of the teeth and a higher CHX dose. The tray method has the advantage of providing complete and consistent coverage of the teeth, especially for subjects who are unable to rinse for 1 minute.
2.3.5 Gel summary

Twice a day application of 1% CHX gel is effective for reducing plaque and bleeding. ⁹⁻¹¹ Using a tray further enhances the effects of CHX gel on the clinical signs of gingivitis because of better and consistent coverage.¹² ⁶⁹ However CHX gel still causes tooth staining.⁹⁻¹¹ Further studies explore alternative CHX application methods.

2.4 Chlorhexidine Spray

2.4.1 Rationale for use and optimal dose

CHX can also been applied with a non-aerosol spray for subjects who are unable to rinse or are uncooperative.¹³ ¹⁵ ⁷⁰⁻⁷³ The optimal dose for plaque inhibition by CHX spray is 6 mg per day,⁷⁰ although volumes of 1.4 to 2.0 ml per day are effective for reducing plaque scores.⁶⁹ ⁷²

2.4.2 Short term studies

2.4.2.1 Compared to chlorhexidine rinse and gel

Francis et al. (1987) compared 0.2% CHX spray, 0.2% CHX rinse and 1% CHX gel in trays and found that the CHX spray was just as effective as the rinse in reducing plaque and bleeding.⁶⁹ Kalaga et al. (1989) also demonstrated that twice a day spraying with 0.2% CHX had the same effect on plaque and bleeding as 0.2% CHX rinse.⁷⁰
2.4.2.2 Compared to stannous fluoride

Chikte et al. (1991) compared 0.2% stannous fluoride spray and 0.2% CHX spray with no mechanical interventions on plaque scores in handicapped children.14 The CHX spray was statistically significant for reducing plaque compared to the fluoride spray (p < 0.05).14

2.4.2.3 Compared to tooth brushing

When 0.2% CHX spray was used as an adjunct to mechanical oral hygiene procedures, there were statistically significant reductions in plaque and bleeding compared to tooth brushing and placebo.73 Burtner et al. (1991) also demonstrated that 0.12% CHX spray in addition to nurse administered tooth brushing twice a day on institutionalized subjects had statistically significant reductions on plaque (p = 0.002), gingival inflammation (p = 0.02) and bleeding (p = 0.03) compared to placebo spray.13

2.4.3 Spray summary

Spraying 0.2% CHX effectively reduces plaque and bleeding without causing noticeable tooth staining,13-15,69,72,73 possibly because of the lower dose.21,57 Spraying may also reduce CHX's desquamate side effect because the method focuses the CHX onto a circumscribed area.71 Although applying CHX by spray is the easiest method for caregivers to provide gingivitis treatment for their patients, it may not sufficiently reach the posterior areas in the mouth.
2.5 Other Methods

2.5.1 Implantable devices

2.5.1.1 Rationale for use

A novel method for applying CHX is the professional dental placement of slow-release CHX chips.\textsuperscript{5,51} The CHX chip is excellent for non-compliant patients or patients with one or two persistently inflamed sites because it is placed by a dentist and only in the sites that require it.

2.5.1.2 Short-term study

Soskolone \textit{et al.} (1997) compared a biodegradable chip containing 2.5 mg of CHX to scaling and root planing in periodontal pockets at baseline and three months.\textsuperscript{5} Reductions in probing depths were statistically significant in sites treated with the CHX chip compared to scaling and root planning alone (1.16 mm ± 0.058 versus 0.70 mm ± 0.056, \(p < 0.0001\), respectively).\textsuperscript{5} Reductions in gingival index scores were also statistically significant in CHX chip treated sites, but plaque and bleeding reductions were negligible compared to the control sites. Since the CHX chips were placed subgingivally, tooth staining was not apparent.
2.5.2 Toothpastes

2.5.2.1 Rationale for use

Since most individuals use a toothbrush and toothpaste to remove dental plaque \(^{74}\) incorporating CHX into toothpaste may be an easy substitution to enhance patient compliance.

2.5.1.2 Short-term studies

Gjermo and Rölla (1971) compared a 0.8% CHX with inorganic abrasives toothpaste, a 0.6% CHX with polymer particles toothpaste, and a placebo toothpaste and demonstrated a statistically significant reduction in mean plaque scores for the CHX toothpastes compared to the placebo.\(^6\) The CHX remained active and stable for up to six months in the toothpastes.\(^6\)

Sanz et al. (1994) investigated a toothpaste containing 0.4% CHX and 0.34% zinc and found the toothpaste effective for reducing plaque and gingivitis compared to a placebo toothpaste.\(^{47}\) Yates et al. (1993) also demonstrated a statistically significant reduction in plaque, gingivitis, and bleeding with a toothpaste containing 1000 parts per million sodium fluoride and 1% CHX.\(^{50}\) The CHX toothpastes developed and tested by Gjermo and Rölla (1971), Sanz et al. (1994), and Yates et al. (1993) all increased supragingival calculus formation and tooth staining.\(^6, 47, 50\)
2.5.2.3 Compared to chlorhexidine rinse

Jenkins et al. (1990) experimented with 13 combinations of CHX and other active ingredients in a dentifrice form and compared these with water and 0.2% CHX mouth rinse to determine their anti-bacterial effects. Although all the toothpaste combinations showed some anti-bacterial effects, the toothpastes were only able to reduce the subjects' salivary bacterial counts for five hours compared to the seven hours of the 0.2% CHX mouth rinse. CHX toothpastes also only reduced bacterial counts by 35% compared to the 70% of CHX mouth rinses.

Since there is little clinical benefit for using CHX toothpastes because of the reduced antimicrobial effect, and increased tooth staining and calculus formation other oral health products were studied.

2.5.3 Dental flosses

2.5.3.1 Rationale for use

Dental floss is another oral self-care aid that is familiar to most individuals. Dental floss is an effective mechanical method for treating and preventing interproximal gingivitis. Although there are many different types of dental floss available, CHX or other antimicrobial agents are not found on commercially available dental floss.

2.5.3.2 Short-term study

The study by Kinane et al. (1992) combined the mechanical properties of dental floss with the chemical properties of CHX in an attempt to determine if this combined product
would be better at reducing gingival bleeding than dental floss alone. In this double-blinded study, a floss holder was designed to deliver 25 μl of 0.1% CHX or placebo into each interdental embrasure while the floss was in position interdentally. The percentage bleeding reduction after two weeks was as follows: 38.3% for conventional floss, 51.5% for the flossing device with CHX, and 51.4% for the flossing device with placebo. The lack of significant inter-group differences was attributed to insufficient daily dose of CHX; 0.25 to 0.50 mg per day compared to the optimal dose of 6 mg per day of sprayed CHX. Although higher concentrations, volumes or twice a day usage may have improved the study results, another explanation is that the floss was blocking the interproximal site and prevented the CHX from reaching the interproximal areas. Kinane et al.'s (1992) study was the premise for my thesis and RCT.

2.6 Conclusion

Concentrations of CHX that are lower than the gold standard of 0.2% can effectively inhibit plaque formation and reduce the bleeding and inflammation associated with gingivitis. Numerous modes of delivery have been explored to optimize the anti-plaque and anti-gingivitis properties of CHX while at the same time, controlling or eliminating its unwanted side effects. Although effective at reducing plaque and bleeding, CHX mouth rinses, whether 2% or lower, gels, and toothpastes continue to cause tooth staining. CHX chips do not cause tooth staining but require placement by a dentist and therefore, are not consumer friendly. Spraying CHX may be an effective method for
treating gingivitis without the tooth staining side effect, but it may not reach all areas in
the oral cavity. A promising approach is the application of CHX via dental floss. CHX
via dental floss could incorporate the mechanical plaque removing property and
accessibility of dental floss with the anti-plaque and anti-gingivitis effects of CHX.
Consumers are familiar with dental floss; therefore, individuals requiring treatment for
gingivitis may accept the addition of CHX on their dental floss. A RCT, which can be
found in Chapter 3, was conducted to test the efficacy of CHX applied by dental floss on
the clinical signs of interproximal gingivitis.
2.7 References


3 THE EFFECTS OF FLOSSING WITH A CHLORHEXIDINE SOLUTION ON INTERPROXIMAL GINGIVITIS: A RANDOMIZED CONTROLLED TRIAL*

3.1 Introduction

Gingivitis is an inflammatory response of the gingivae to the bacterial challenge in dental plaque.\(^1\)\(^-\)\(^3\) Although gingivitis can occur on all gingival surfaces, it is more prevalent in the interproximal areas.\(^4\) Gingivitis can be treated by mechanically removing the dental plaque such as, tooth brushing and dental flossing\(^4\)\(^-\)\(^6\) or by chemically inhibiting plaque via chlorhexidine (CHX).\(^7\)\(^-\)\(^9\)

Kinane \textit{et al.} (1992) investigated a novel flossing device that combined the beneficial aspects of dental floss and CHX to reduce gingival bleeding in gingivitis subjects.\(^10\) No significant differences were found between the CHX and placebo flossing devices.\(^10\) Although the dose of CHX may have been too low,\(^10\) another explanation is that the dental floss blocked the CHX from reaching the interproximal areas.

The purpose of this three-month, double-blinded, parallel randomized controlled trial (RCT) was to determine whether dental floss immersed in CHX would reduce the clinical signs of interproximal gingivitis better than a floss in placebo solution. Since CHX is known to cause extrinsic brown tooth stain,\(^11\) a secondary aim was to determine whether CHX applied via dental floss resulted in tooth staining.

* A version of this chapter will be submitted for publication. Imai PH, Putnins EE, Brunette DM. The effects of flossing with a chlorhexidine solution on interproximal gingivitis: a randomized controlled trial. CJDH.
3.2 Materials and Methods

The study received approval from the University of British Columbia’s Clinical Research Ethics Board (#C05-0513 & H05-70513). (See Appendix A.6)

3.2.1 Subjects

Twenty-seven adults with gingivitis or early to moderate, localized periodontitis were recruited from Vancouver, British Columbia through newspaper advertisements, community advertisements, and word of mouth. The American Academy of Periodontology (1999) definitions of gingivitis and early and moderate localized periodontitis were used in the study. Gingivitis is defined as gingival inflammation that is confined to the gingivae with no clinical attachment loss or on stable, but reduced periodontium. Early periodontitis is defined as probing depths of 3-4 mm with 2-3 mm clinical attachment loss and moderate periodontitis is defined as probing depths of 5-7 mm with clinical attachment loss of 4-6 mm. Localized periodontitis is defined as clinical attachment loss in less than 30% of the total sites.

3.2.1.1 Inclusion/Exclusion criteria

Subjects were enrolled if they were non-smoking adults with gingivitis or localized periodontitis. A minimum of 10 bleeding on probing sites was required. Subjects who accepted were required to floss daily, attend all instructional sessions, and sign a consent form (See Appendix A.2).

Subjects were excluded from the study if they were pregnant or planned to become pregnant within the next 3 months, were allergic to CHX or quinine sulfate (QS), or were
required to take antibiotic pre-medication for dental treatment. Subjects were also excluded if they had full or partial dentures, extensive crown and bridge coverage, full orthodontic bands and brackets, or advanced generalized periodontitis (i.e., 30% or more sites having clinical attachment loss of greater than 7 mm). Subjects were excluded or removed from the study if they took antibiotics, Dilantin, Cyclosporin A, Nifedipine or other calcium channel blockers, daily aspirin or anti-coagulants, CHX or whitening products.

3.2.1.2 Randomization

The enrolled subjects were randomly assigned to treatment group (CHX), dental floss (Johnson & Johnson Reach® unflavoured waxed dental floss, Montreal, Canada) with 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona), or placebo group (QS), dental floss (Johnson & Johnson Reach® unflavoured waxed dental floss, Montreal, Canada) with 0.1% quinine sulfate solution. The placebo solution was prepared by a pharmacist to taste, smell, and look like the CHX solution. Subjects were randomized using a block design determined by an individual, who was not involved with the study in any other capacity. Subjects were enrolled on an ongoing basis between March 2006 and mid-September 2006, at which time the study was closed to accrue to allow the subjects to complete the 3 month study prior to the mid-December completion date.
3.2.2 Study visits

3.2.2.1 Screening visit

The study consisted of 4 visits over a 3 month period. All potential subjects underwent a screening visit at which medical and dental histories were recorded (See Appendix A.3), periodontal condition and number of bleeding points was assessed, and the subject was informed about the nature of the study. If the subject met the inclusion and exclusion criteria, informed consent was obtained and the subject was scheduled for the debridement appointment.

3.2.2.2 Debridement visit (Week -1)

During the debridement appointment (Week -1) calculus and plaque were removed with a combination of ultrasonic and hand instrumentation. Superficial tooth stains were removed with rubber cup prophylaxis and pumice. Flossing technique was reviewed until the subject was adept at using dental floss. Additional flossing instructions were available on a video clip on the study website and in the flossing diary (See Appendix A.5). Subjects were requested to brush as usual, but refrain from using electric toothbrushes. Mouthwashes and additional professional dental hygiene services such as scaling, root planing, and rubber cup polishing were also prohibited during the study period.

3.2.2.3 Baseline visit (Week 0)

Approximately one week after the debridement visit, subjects returned for baseline data collection (Week 0) (See Appendix A.4), which was collected in the following order:
gingival index (GI),\textsuperscript{13} stain index (SI), plaque index (PI),\textsuperscript{14} probing depths (PD) with bleeding on probing (BOP).

At the end of the baseline visit, subjects received a randomly assigned floss and flossing diary (See Appendix A.5) to record their flossing activity. Subjects were instructed to brush as usual then floss once a day with approximately 18” of dental floss. They were also requested not to rinse their mouth with water after flossing to prevent the “medicine” from being washed away.

The floss container, which was full of solution, was placed in a heavy glass candleholder to prevent accidental spillage (See Figure 3.1). Subjects were requested to ensure that the dental floss was wet at all times and were given a small bottle with extra solution to refill the floss container as needed. (See Figure 3.1) If the subject thought the floss was getting dry while flossing, he or she was encouraged to use two pieces of dental floss, i.e., one piece for the “top” teeth and one for the “bottom” teeth.

All subjects received an Oral-B soft Indicator\textsuperscript{®} toothbrush #40 (Gillette Co., Boston, Massachusetts) and Colgate\textsuperscript{®} regular anti-cavity mint toothpaste (Colgate-Palmolive Canada Inc., New York, New York) with instructions to only use these products with their assigned dental floss and not to share the study materials with family members.
3.2.2.4 Follow-up visits: Weeks 6 and 12

Measurements were retaken on the same teeth in the same order as Week 0. The dental floss, floss diary, toothbrush, and toothpaste were replenished with a new supply at Week 6. Subjects were questioned about any changes in their medical histories and if they had experienced any side effects at each of the follow-up visits. To assess flossing compliance, the length of remaining dental floss in the container was measured and compared with the self-reported usage recorded in the flossing diary. If, at the end of the study, a subject presented with calculus, stain, and/or bleeding on probing, an exit debridement was performed. All subjects were dismissed at Week 12 and requested to return to their usual oral health care professional for continuing care.
3.2.3 Measurements

One examiner, who was blinded to the treatment assignments and calibrated before the study began, collected the clinical data on all subjects. The measurements were taken on six sites per tooth (mesial-buccal, buccal, distal-buccal, distal-lingual, lingual, and mesial-lingual) on all teeth except third molars and teeth with crown and bridge coverage. Index scores were averaged per tooth then added together and divided by number of teeth for the full mouth score. Teeth were lightly dried with pressurized air prior to the measurements. Indices are as follows:

1) Gingival Index (GI), Modified Løe and Silness (1963)\textsuperscript{13}
   
   0 = No inflammation
   1 = Mild inflammation- slight change in colour, little change in texture
   2 = Moderate inflammation- moderate glazing, redness, edema, and hypertrophy
   3 = Severe inflammation- marked redness and hypertrophy, tendency for spontaneous bleeding, ulceration

2) Stain Index (SI)
   
   0 = No visible stain
   1 = Light, barely visible stain
   2 = Moderate stain
   3 = Heavy stain
3) **Plaque Index (PI), Modified Silness and Løe (1964)**

Teeth were disclosed with Trace® disclosing solution (Young dental manufacturing, Earth City, MO, USA) and lightly rinsed.

- 0 = No plaque
- 1 = Light film of plaque
- 2 = Moderate accumulation of plaque
- 3 = Heavy accumulation of plaque

4) **Probing Depths (PD), in millimeters**

A pressure-sensitive, 3-6-9 mm periodontal probe with a point tip diameter of 0.5 mm (Kerr-Hawe Click-Probe®, Kerr U.S.A. 1717 West Collins Avenue, Orange, CA 92867) set at 25 N (Newtons) was used to record the PD and BOP.

5) **Bleeding on Probing (BOP)**

BOP was collected in conjunction with probing depths. BOP was noted within 15 seconds of probing. In sites of profuse bleeding, the area was rinsed with water and air dried lightly.

- 1 = Presence of bleeding
- 0 = Absence of bleeding

3.2.4 **Statistical Analyses**

An intention-to-treat protocol and full mouth scores were used in the statistical analyses. Student t-tests were used for between treatment and within treatment analyses. For data
that was not normally distributed, Wilcoxon-Rank Sum and Wilcoxon Signed-Rank tests were conducted. Alpha was set at 5%. Post-hoc exploration of the data was done using stratification, analysis of covariance (ANCOVA) with baseline values as a covariate.

3.3 Results

The examiner's intra-examiner kappa statistic was 0.65 for GI, 0.96 for PI, and 0.36 for BOP.

Twenty-six (18 women and 8 men) of the 27 enrolled subjects completed the 12 week study. One subject withdrew at Week 6 because she was unable to "get into the flossing habit." The subject flossed for 8 days immediately after being randomized and then ceased flossing prior to the Week 6 visit. Another subject, who was on an extended holiday, missed the Week 6 visit, but continued to follow the research protocol and presented at Week 12.

The subjects and clinical examiner reported no side effects. Subjects from both groups commented that their mouths felt "cleaner", "better", "fresher," and they no longer noticed any bleeding when brushing and flossing.

3.3.1 Week 0

At Week 0, the two groups were clinically similar for GI, PI, SI, PD, and BOP. Slight mean differences between groups were not statistically significant with Student's t-test. Nevertheless, to control for the possibility of these differing baseline values on the
outcomes, ANCOVA was conducted using the baseline values as a covariate. The adjusted p-values are reported in addition to the p-values from the Student t-tests.

### 3.3.2 Probing Depths (PD)

A statistically significant reduction in probing depths was found for the subjects using the floss presoaked with CHX compared to those using the floss presoaked with the placebo solution at Week 6 (p = 0.029, adjusted p-value = 0.022). At Week 12, the mean PD for subjects using the floss presoaked in CHX remained below baseline values compared to those using the floss presoaked in QS, which rose above its baseline value; however, this was not statistically significant (p = 0.177, adjusted p-value = 0.256).

Since dental floss is more effective in probing depths that are less than 4 mm, further analyses were conducted with the subjects’ gingival sites stratified into PD < 4mm and PD ≥ 4 mm. At Week 6, there was a statistically significant reduction in PD in sites that were originally < 4mm for subjects using the CHX soaked floss compared to those using the QS soaked floss (p = 0.006, adjusted p-value = 0.034), but not in sites that were initially ≥ 4 mm (p = 0.73, adjusted p-value = 0.50) (See Table 3.1).

Table 3.1: Comparison of chlorhexidine (CHX) and placebo (QS) groups with sites stratified into probing depths < or ≥ 4 mm at Week 6.

<table>
<thead>
<tr>
<th>Initial probing depths (PD)</th>
<th>CHX group (n = 12)</th>
<th>QS group (n = 14)</th>
<th>p-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD &lt; 4mm</td>
<td>2.06 ± 0.12</td>
<td>2.23 ± 0.16</td>
<td>0.006†</td>
<td>0.034‡</td>
</tr>
<tr>
<td>PD ≥ 4 mm</td>
<td>3.73 ± 1.18</td>
<td>4.05 ± 0.09</td>
<td>0.73*</td>
<td>0.50*</td>
</tr>
</tbody>
</table>

† 2 sample t-test, ‡ ANCOVA with baseline as covariate, * Wilcoxon Rank Sum test, • Wilcoxon Rank Sum test on change from baseline scores.
At Week 12, the shallow sites continued to demonstrate a statistically significant reduction in PD for the CHX group but not for the QS group (p = 0.005, adjusted p-value = 0.011) (See Table 3.2). There was no statistically significant difference for PD between the CHX and QS groups for the sites that were initially ≥ 4 mm (p = 0.85, adjusted p-value 0.32) (See Table 3.2).

Table 3.2: Comparison of chlorhexidine (CHX) and placebo (QS) groups with sites stratified into probing depths < or ≥ 4 mm at Week 12.

<table>
<thead>
<tr>
<th>Initial probing depths (PD)</th>
<th>CHX group (n = 12)</th>
<th>QS group (n = 14)</th>
<th>p-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>PD &lt; 4 mm</td>
<td>2.09</td>
<td>0.16</td>
<td>2.26</td>
<td>0.11</td>
</tr>
<tr>
<td>PD ≥ 4 mm</td>
<td>3.76</td>
<td>1.13</td>
<td>4.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>

† 2 sample t-test, ‡ANCOVA with baseline as covariate, *Wilcoxon Rank Sum test, • Wilcoxon Rank Sum test on change from baseline scores

3.3.3 Bleeding on Probing (BOP)

A statistically significant reduction for BOP (mean change of −0.04) occurred for all subjects (p = 0.017) from Week 0 to Week 6. At Week 6, there was no statistically significant difference between the CHX and QS groups (p = 0.596, adjusted p-value = 0.913).

Reductions in BOP continued from Week 0 to Week 12 for all subjects (mean change of −0.02), but this was not statistically significant (p = 0.179, 95% CI −0.06, 0.01). Of the initial positive bleeding sites, 83% stopped bleeding in the CHX group and 78% in the
QS group. At Week 12, there was no statistically significant difference between the CHX and QS groups (p = 0.662, adjusted p-value = 0.761).

Since it would be easier to discern a larger magnitude of change of disease to health, further analyses were conducted with the subjects stratified according to “mild gingivitis” (defined for the purposes of this RCT as < 11 initial positive BOP sites, which was the minimal number of BOP sites to be considered for inclusion into the RCT) and “moderate gingivitis” (≥ 11 initial positive BOP sites). Subjects with moderate gingivitis who used the floss presoaked in CHX had a statistically significant reduction in BOP from Week 0 to Week 6 (p = 0.0078) (See Table 3.3), but subjects with the mild gingivitis did not (p = 0.50). Subjects with moderate gingivitis who were using the floss presoaked in QS also had a reduction in BOP from Week 0 to Week 6, but this did not reach statistical significance (p = 0.063) (See Table 3.3). There was no statistically significant reduction in BOP for subjects with mild gingivitis using the floss with QS (p = 0.73) (See Table 3.3).

Table 3.3: Comparison of mean change in bleeding on probing (BOP) from Week 0 to Week 6 for subjects stratified according to mild gingivitis (< 11 initial BOP sites) and moderate gingivitis (≥ 11 initial BOP sites) using floss soaked in either chlorhexidine (CHX) or placebo (QS).

<table>
<thead>
<tr>
<th>Gingivitis severity (initial BOP sites)</th>
<th>Floss used</th>
<th>N</th>
<th>Mean change from Week 0 to Week 6</th>
<th>SD</th>
<th>P-value Wilcoxon Signed Rank test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate (≥ 11)</td>
<td>CHX</td>
<td>8</td>
<td>-0.11625</td>
<td>0.0884691</td>
<td>0.0078</td>
</tr>
<tr>
<td>Moderate (≥ 11)</td>
<td>QS</td>
<td>6</td>
<td>-0.078333</td>
<td>0.0611283</td>
<td>0.063</td>
</tr>
<tr>
<td>Mild (&lt;11)</td>
<td>CHX</td>
<td>4</td>
<td>0.0225</td>
<td>0.0403113</td>
<td>0.50</td>
</tr>
<tr>
<td>Mild (&lt;11)</td>
<td>QS</td>
<td>8</td>
<td>0.01875</td>
<td>0.0566789</td>
<td>0.73</td>
</tr>
</tbody>
</table>
At Week 6, Wilcoxon Rank-Sum tests demonstrated no statistically significant difference between the CHX and QS groups regardless of whether the subjects had mild gingivitis (p = 0.37, adjusted p-value = 0.46) or moderate gingivitis (p = 0.45, adjusted p-value = 0.49).

Subjects with moderate gingivitis continued to demonstrate reductions in BOP scores from Week 0 to Week 12, but this did not reach statistical significance for either group (CHX soaked floss, p = 0.086; QS soaked floss, p = 0.063). There were also no statistically significant reductions in BOP for subjects with mild gingivitis whether they used the floss presoaked in CHX (p = 0.99) or the floss presoaked in QS (p = 0.16).

At Week 12, there were no statistically significant differences for BOP between the CHX and the QS groups, regardless of whether the subjects had mild gingivitis (p = 0.29, adjusted p-value = 0.62) or moderate gingivitis (p = 0.66, adjusted p-value = 0.35).

Since it is easier for subjects to floss the anterior teeth (canine to canine) as opposed to the posterior teeth (first premolar to second molar), further analyses were conducted with the BOP sites separated into anterior and posterior areas. Statistically significant reductions in BOP occurred from Week 0 to Week 6 for subjects who were using the CHX soaked floss in both anterior (p = 0.008) and posterior areas (p = 0.036). The subjects using the QS soaked floss did not demonstrate a statistically significant reduction in BOP for anterior (p = 0.398) or posterior areas (p = 0.064) (See Table 3.4). At Week 6, there was no statistically significant difference for BOP between the CHX and QS groups.
for anterior areas (p = 0.425, adjusted p = 0.174) and posterior areas (p = 0.239, adjusted p = 0.659).

Table 3.4: Comparison of the mean changes in bleeding on probing (BOP) for sites stratified according to anterior or posterior areas of the mouth in subjects using either the floss soaked in chlorhexidine (CHX) or placebo from Week 0 to Week 6.

<table>
<thead>
<tr>
<th>Area in subjects’ mouth</th>
<th>Floss used</th>
<th>N</th>
<th>Mean change</th>
<th>SD</th>
<th>P-value Paired t-tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior (canine to canine)</td>
<td>CHX</td>
<td>12</td>
<td>- 0.019</td>
<td>0.001</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>QS</td>
<td>13</td>
<td>- 0.001</td>
<td>0.0004</td>
<td>0.398</td>
</tr>
<tr>
<td>Posterior (bicuspids to molars)</td>
<td>CHX</td>
<td>12</td>
<td>- 0.017</td>
<td>0.002</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>QS</td>
<td>13</td>
<td>- 0.008</td>
<td>0.0003</td>
<td>0.064</td>
</tr>
</tbody>
</table>

From Week 0 to Week 12, statistically significant reductions in BOP continued to occur in the anterior areas for the subjects using the CHX soaked floss (p = 0.011); the posterior areas no longer had a statistically significant reduction (p = 0.227) (See Table 3.5).

Subjects using the QS soaked floss did not demonstrate a statistically significant reduction in BOP from Week 0 to Week 12, regardless of whether the sites were in the anterior (p = 0.091) or posterior (p = 0.318) regions of their mouths (See Table 3.5). At Week 12, there was no statistically significant difference for BOP between the CHX and QS groups for anterior areas (p = 0.537, adjusted p = 0.910) and posterior areas (p = 0.794, adjusted p = 0.726).
Table 3.5: Comparison of the mean changes in bleeding on probing (BOP) for sites stratified according to anterior or posterior areas of the mouth in subjects using either the floss soaked in chlorhexidine (CHX) or placebo from Week 0 to Week 12.

<table>
<thead>
<tr>
<th>Area in subjects' mouth</th>
<th>Floss</th>
<th>N</th>
<th>Mean change</th>
<th>SD</th>
<th>P-value Paired t-tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior (canine to canine)</td>
<td>CHX</td>
<td>13</td>
<td>-0.015</td>
<td>0.001</td>
<td>0.012</td>
</tr>
<tr>
<td>Anterior (canine to canine)</td>
<td>QS</td>
<td>14</td>
<td>-0.008</td>
<td>0.0004</td>
<td>0.091</td>
</tr>
<tr>
<td>Posterior (bicuspids to molars)</td>
<td>CHX</td>
<td>13</td>
<td>-0.007</td>
<td>0.001</td>
<td>0.227</td>
</tr>
<tr>
<td>Posterior (bicuspids to molars)</td>
<td>QS</td>
<td>14</td>
<td>0.002</td>
<td>0.0003</td>
<td>0.318</td>
</tr>
</tbody>
</table>

3.3.4 Plaque Index

Although the subjects using the CHX soaked floss appeared to have constant plaque scores compared to the slightly increasing scores in the QS group from Week 0 to Week 6 as well as from Week 0 to Week 12 (See Figure 3.2), there was no significant difference in mean plaque scores between the CHX and QS groups at Week 6 (p= 0.937, adjusted p-value = 0.540) or Week 12 (p = 0.497, adjusted p-value 0.107).
3.3.5 Gingival Index

Both the CHX and QS groups had statistically significant reductions in mean GI scores from Week 0 to Week 6 (mean change of −0.56, p < 0.001) as well as from Week 0 to Week 12 (mean change of −0.58, p < 0.0001) (See Figure 3.3). There was no statistically significant difference between the CHX and QS groups at Week 6 (p = 0.628, adjusted p-value = 0.693) or Week 12 (p = 0.725, adjusted p-value = 0.674).
3.3.6 Stain Index

There was no statistically significant difference between the CHX and QS groups for stain at Week 6 (p = 0.907, adjusted p = 0.516) or Week 12 (p = 0.181, adjusted p-value = 0.315). Both groups had a slight, but not statistically significant, increase in stain over the 12 weeks (mean change 0.05, p = 0.136).

3.3.7 Flossing Compliance

At Week 6, the reported median flossing compliance was 97.50% (IQR 87.00, 100.00) for subjects using CHX soaked floss and 96.50% (IQR 86.00, 100.00) for subjects using QS soaked floss, which was not statistically different. Median yards of floss used were
42.50 (IQR 26.00, 51.00) for the CHX group and 35.50 (IQR 23.00, 59.00) for the QS group, which was not statistically different.

At Week 12, the reported median flossing compliance was 100.00% (IQR 88.00, 100.00) for the CHX group and 93.00 (IQR 76.00, 100.00) for the QS group, which was not statistically different. Median yards of floss used were 43.00 (IQR 33.00, 52.00) for the CHX group and 43.00 (IQR 30.00, 50.00) for the QS group. Twenty-one yards was the expected amount of floss to be used in 42 days, based on 18” per day.

3.4 Discussion

The introduction of a daily flossing regimen resulted in an overall benefit for all study subjects. Flossing, as shown by the results of the positive control group, resulted in statistically significant reductions in BOP scores from Week 0 to Week 6, and continued to show reductions in bleeding to a lesser degree up to Week 12. All subjects also had statistically significant reductions in GI scores over the 12 week study. The reductions in bleeding and gingival index scores found in this RCT are similar to the results found in other studies, which have demonstrated the beneficial effects of flossing for the treatment of gingivitis.15-18

However, presoaking the dental floss in CHX solution had additional benefits compared to the floss in the placebo solution. The CHX soaked dental floss had statistically significant reductions for probing depths in sites that were initially less than 4 mm compared to the floss in placebo solution, which did not demonstrate any statistically
significant probing depth reductions. Both groups did not have statistically significant reductions in probing depths for sites that were initially 4 mm or more because dental floss can deplaque sulcular depths to a maximum of 3 mm.\textsuperscript{15,19,20} The CHX soaked floss may have been able to carry the CHX into the interproximal area to produce a reduction in probing depths similar to the effects seen by oral irrigation with CHX solution. For example, Flemmig \textit{et al.} (1990) demonstrated a reduction in probing depths (mean reduction of 4.6\% at 6 months, p < 0.05) in shallow sulci by irrigating with 0.06\% CHX rinse.\textsuperscript{21} Although the application method differs, oral irrigation may flush CHX subgingivally into the sulcus\textsuperscript{21} just as dental floss may carry CHX into the sulcus to reduce probing depths.

The CHX soaked floss also demonstrated additional BOP reductions for subjects with 11 or more initial BOP sites compared to the QS soaked floss. The subgroup of moderate gingivitis subjects using the CHX soaked floss had a statistically significant reduction in BOP from Week 0 to Week 6, which continued to a lesser degree up to Week 12. Although the subjects with 11 or more initial BOP sites in the QS group also had reductions in BOP from Week 0 to Week 12, this did not reach statistical significance, indicating that the CHX was having an additional effect over and above the mechanical effects of flossing on the bleeding sites. CHX mouth rinse has been shown in other studies to reduce bleeding, with reductions ranging from 46.0\% to 67.0\%.\textsuperscript{8,9,16,21-25} According to Cumming and L\öe (1973) and Caton \textit{et al.} (1993) CHX mouth rinses may have limited effects interproximally,\textsuperscript{4,26} but by using a CHX soaked dental floss we may
have been able to carry the CHX into the interproximal area to exert an additional positive effect on the interproximal bleeding scores.

Since individuals can floss the anterior teeth (canine to canine) more effectively than the posterior teeth (first premolar to second molar), analyses were conducted with the BOP sites grouped into anterior and posterior sites. Although the subjects using the CHX soaked floss had statistically significant reductions in BOP from Week 0 to Weeks 6 and 12 for the anterior areas, the QS group did not. The posterior areas had a statistically significant reduction in BOP from Week 0 to Week 6 for the CHX group, but this did not persist to Week 12. Our results are similar to Wong and Wade (1985) in that the subjects were able to floss the anterior teeth more effectively than the posterior teeth. Also, although flossing reduced BOP in both groups as stated earlier, it is apparent that the addition of CHX to the floss enhanced the reduction in BOP scores in specific sites over time because the QS group did not demonstrate any statistically significant changes in specific sites of BOP within the mouth.

The plaque index results further collaborates that it may not necessarily be only plaque removal that is having an effect on bleeding scores. Dental floss exerts its beneficial effect on inflamed gingival tissues by removing dental plaque, which is the primary etiological cause of plaque-induced gingivitis. Therefore, one would expect a reduction in plaque index scores to correspond with the reductions in BOP and GI scores. According to Carr et al. (2000) flossing alone effectively reduces plaque by 65%.
However, our RCT did not demonstrate statistically significant reductions in plaque scores for the CHX or QS groups. The data trends indicated a slight increase in mean plaque scores for the QS group, but fairly constant scores for the CHX group. Since there were no statistically significant differences between the CHX and QS groups for PI and compliance scores, the subjects were fairly equivalent in terms of flossing effectiveness and frequency.

Although there is the possibility that the floss with the CHX was exerting some anti-plaque effects, which is a property of CHX, the amount of CHX being retained intraorally may have been too low to demonstrate a statistically significant effect. CHX is concentration-dependent, which means more CHX is retained intraorally with higher and higher concentrations. Since CHX has substantivity, which is the ability of a drug to be adsorbed then slowly released in active form over time, it has a prolonged bacteriostatic effect. However, in order for CHX to exert its bacteriostatic effect sufficient amounts of CHX must first be retained intraorally. In our RCT, only 0.12% CHX was used in small volumes on the dental floss, which may not have been sufficient for optimal intraoral retention and subsequently, statistically significant anti-plaque effects.

There is also the possibility that all the subjects’ overall flossing techniques were not very effective and hence, the plaque scores did not decrease even with repeated daily practice over the 12 weeks. Although all the subjects received flossing instructions and demonstrated their flossing technique at the debridement visit with written flossing instructions available in their flossing journals and a video clip available on the study.
website, it is possible that a one time, face-to-face session on flossing techniques is insufficient for the subjects to master effective interproximal plaque control.

The other benefit of using a CHX soaked dental floss rather than a CHX mouth rinse is minimal tooth staining. CHX mouth rinse is known to cause tooth staining within a few days of use in 3 out of 4 individuals who use it and this is the primary reason for low compliance with the CHX mouth rinse regimen. However, in our RCT there was no noticeable tooth staining in subjects applying the CHX with dental floss. Both the CHX and QS groups had slight increases in tooth stain over the 12 weeks but this was not statistically significant and could be attributed to dietary sources such as tea and coffee drinking.

Subjects’ compliance with the flossing regime was excellent, with most subjects flossing daily. Although the subjects used more than twice the amount of floss than was expected, the high usage corresponded to the high numbers of self-reported flossing days, indicating that compliance was high. Subjects may have followed the recommendation of using two pieces of floss per flossing event to ensure that all areas of the mouth received the “medicine.”

3.5 Conclusion

Dental flossing reduces gingival index and bleeding on probing scores and is an effective method for treating gingivitis. However, dental floss presoaked in a 0.12% CHX solution offers additional benefits for the treatment of gingivitis such as, reducing probing depths
in shallow sulcular sites and bleeding in subjects with moderate amounts of gingival bleeding. The floss with CHX is also more effective for reducing bleeding in all areas of the oral cavity, but more so in the anterior sites, than the floss with the QS. There was no statistically significant reduction in plaque levels for either group, possibly indicating ineffective flossing technique rather than lack of compliance since both groups had high levels of flossing compliance as indicated by self-reports and yards of floss used. Since the CHX soaked dental floss improves some clinical periodontal indices without tooth staining, subject compliance with the CHX soaked floss may be higher than with CHX mouth rinse regimen.

3.5.1 Future Studies

Future studies may consider including subjects with moderate to severe gingivitis since reductions in gingival bleeding were seen in subjects with more bleeding sites than fewer bleeding sites. Increasing the concentration of CHX may also increase the effect size because CHX is concentration-dependent. \(^{11}\) In our RCT, the 0.12% concentration may not have been sufficient, especially with the amount that was carried on the dental floss, to allow enough CHX to be retained intraorally for maximum anti-plaque and anti-gingivitis effects. Other studies could explore different interproximal aids that are available on the market since there is the possibility that CHX was lost during handling of the manual floss or apply other anti-microbial agents via dental floss for the treatment of gingivitis.
3.6 References


4 Conclusions

4.1 Overall thesis conclusions

Gingivitis is the inflammatory response of the host’s gingival tissues to the bacterial challenge of dental plaque.\textsuperscript{1} Removing the dental plaque via dental flossing or inhibiting the plaque via chlorhexidine will reverse the clinical signs of gingivitis.\textsuperscript{1,2} (See Chapters 1 and 2). In this thesis, we explored the combined effects of dental floss immersed in chlorhexidine (CHX) for the treatment of interproximal gingivitis. Before the randomized controlled trial (RCT) was conducted, various types of dental floss were tested in-vitro to determine which floss would be the most suitable for carrying the CHX (See Appendix A.1) with the Johnson & Johnson waxed dental floss (Johnson & Johnson Reach\textsuperscript{®} unflavoured waxed dental floss, Montreal, Canada) being chosen for the RCT. The overall results of the RCT demonstrated that presoaking dental floss in a 0.12\% CHX solution provided additional benefits compared to a dental floss in placebo solution for reducing probing depths in shallow sulci and bleeding in subjects with moderate gingivitis without causing the tooth staining that is commonly associated with the use of CHX mouth rinses.

4.2 Future studies

Future studies may explore other options such as enrolling subjects with moderate to severe gingivitis, applying other anti-microbial agents via dental floss, using other interdental aids to apply CHX, or increasing the CHX concentration on the dental floss.
In our RCT, subjects with mild to moderate clinical signs of gingivitis received the intervention. However, it was apparent that the CHX soaked floss had a greater impact on subjects exhibiting moderate clinical signs of gingivitis. According to Trombelli et al. (2004), a larger magnitude of change can be observed in subjects who are classified as "high responders" to the presence of plaque. Subject who were considered high responders by Trombelli et al. (2004) were in the upper interquartile range for gingival crevicular fluid flow for the study population. By increasing the signal to noise ratio, it may be easier to detect changes in the subjects’ gingival condition with clinical periodontal indices.

Since CHX is a prescription drug, dental floss immersed or coated with CHX may not be readily available for the general population. Therefore, other studies may consider applying Listerine® Antiseptic mouth rinse (Pfizer Inc., Morris Plains, NJ, USA), which is a nonprescription essential oil rinse, via dental floss to treat interproximal gingivitis. Listerine® demonstrated statistically significant reductions of 14% for gingival index scores (p < 0.001) and 18.8% for plaque scores (p < 0.001). Although the reductions for Listerine were not as large as those for CHX, which had a gingival reduction of 18.2% and a plaque reduction of 21.6%, its nonprescription status may enhance its availability to the general population.

Other future studies may explore the use of different inter-dental aids to carry the CHX. In our RCT, subjects manually flossed with the CHX floss, which may have resulted in some of the CHX going on their fingers rather than in their gingival sulci. Using Butler
GUM Eez-thru flossers® (Sunstar Americas Inc., Chicago, IL)⁶, Butler GUM Flosbrush® (Sunstar Americas Inc., Chicago, IL)⁶, Oral-B Hummingbird flosser® (Gillette, Boston, Massachusetts)⁷, Butler GUM soft-picks® (Sunstar Americas Inc., Chicago, IL)⁶, or small interdental brushes may carry more CHX into the interproximal areas because the subjects would not touch the surfaces of the aids carrying the CHX.

Note: Although Butler GUM has added CHX to their interdental brushes and toothbrushes, they emphasize that it is not to prevent disease but to control bacterial growth on the brushes.⁶ Having the subjects dip the interdental aid into the CHX solution prior to inserting it inter-proximally may provide additional benefits than just mechanically removing the dental plaque with the same interdental aid. Future studies exploring the use of other interdental aids with CHX are needed.

CHX’s anti-plaque effect is concentration-dependent,²,⁸ (See Chapter 2) therefore, increasing the concentration of CHX on the dental floss or increasing the frequency of CHX flossing per day may increase the effect size. In our RCT, subjects flossed once a day with 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona). Statistically significant effects were demonstrated for probing depths and bleeding, but not for plaque scores. Future studies using a 0.2% CHX or twice a day flossing with 0.12% CHX are needed.
4.3 References


Appendix A

A.1 ASSESSING THE BEST DENTAL FLOSS TO CARRY THE CHLORHEXIDINE IN THE RANDOMIZED CONTROLLED TRIAL: AN IN-VITRO STUDY.

A.1.1 Introduction

There are many different types of dental floss available on the Canadian market: unwaxed, waxed, woven, unwoven, spongy-like, yarn-like, and shred-resistant. In the literature, all types of floss were effective for reducing plaque scores, although the unwaxed dental floss was the least favoured by study subjects. In our randomized controlled trial (RCT), the dental floss would have to carry chlorhexidine (CHX) into the interproximal areas. Anecdotal evidence indicated that the Johnson & Johnson waxed dental floss (Reach, McNeil-PPC Inc., Montreal, Quebec) was capable of carrying CHX into the interproximal areas, but it was unknown if other types of floss would be better. The purpose of the in-vitro study was to determine the best floss for carrying CHX for the RCT.

A.1.2 Materials and Methods

In a series of triplicate in-vitro experiments, the following dental flosses were tested to determine which floss would be best suited for carrying and releasing the CHX: Johnson & Johnson waxed® unflavoured dental floss (Reach, McNeil-PPC Inc., Montreal, Quebec), Oral-B Ultrafloss® (Gillette Inc., Boston, Massachusetts), Oral-B shred-resistant Satin® floss (Gillette Inc., Boston, Massachusetts), Butler GUM unwaxed® woven floss (Sunstar Americas Inc., Chicago, Illinois). Each spool of dental floss was
completely immersed in 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona) for a minimum of 48 hours and a maximum of 6 weeks to mimic the conditions of the RCT.

To test the effects of the CHX soaked dental flosses on oral bacteria a bacterial broth culture was prepared. Using a piece of Ultrafloss® dental floss, a plaque sample was collected from a subject, who allowed plaque to accumulate for 12 hours. Approximately 1” of this used dental floss was placed in a test tube of 5.5 ml of liquid typticase soy and incubated overnight at 34°C to allow bacterial growth. The bacterial broth was diluted by adding 30 μl of the bacterial broth to another test tube of 5.5 ml trypicase soy broth medium. A further dilution was done by adding 10 μl of the first dilution of bacterial broth to another test tube of 5.5 ml trypicase soy broth medium for an approximate dilution of 1/3000. This step was repeated as necessary to provide enough of the bacteria culture for the experiment. Thirty test tubes each containing 90 μl of the final dilution was prepared for the triplicate series.

To test the bacteriostatic effects of the CHX soaked dental flosses, 18” of each CHX soaked dental floss was added to the test tubes containing the 90 μl of bacterial broth for 3 sets of 4 test flosses. To determine if the floss itself contained any substances that may have had any bacteriostatic effects, 18” of each type of dental floss was also tested; 3 sets of 4 control flosses. The control dental flosses were similar to the test flosses but were not immersed in the CHX. The CHX solution was included in the experiment as a positive control. The RCT placebo solution of 0.1% quinine sulfate (QS) was also tested.
to determine if the QS solution would be a suitable placebo, i.e., not have any bacteriostatic effects.

The following dental flosses were used in the study:

**Test flosses:**

1) 18" piece of Oral-B Ultrafloss® (Gillette Inc., Boston, Massachusetts) immersed in 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona)
2) 18" piece of Oral-B Satin® floss (Gillette Inc., Boston, Massachusetts) immersed in 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona)
3) 18" piece of Butler Gum unwaxed® floss (Sunstar Americas Inc., Chicago, Illinois) immersed in 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona)
4) 18" piece of Johnson & Johnson waxed® floss (Reach, McNeil-PPC Inc., Montreal, Quebec) immersed in 0.12% CHX (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona)

**Control flosses:**

5) 18" piece of Oral-B Ultrafloss® (Gillette Inc., Boston, Massachusetts)
6) 18" piece of Oral-B Satin® floss (Gillette Inc., Boston, Massachusetts)
7) 18" piece of Butler Gum unwaxed® floss (Sunstar Americas Inc., Chicago, Illinois)
8) 18" piece of Johnson & Johnson waxed® floss (Reach, McNeil-PPC Inc., Montreal, Quebec)
RCT solutions:

9) 100 µl of 0.12% chlorhexidine (CHX) solution (Peridex®, Zila Pharmaceuticals, Inc., Phoenix, Arizona) (positive control and RCT test solution)

10) 100 µl of 0.1% quinine sulfate (QS) solution (RCT placebo solution)

The tubes were vortexed and then allowed to rest for 2 hours. 200 µl from each tube was then plated onto corresponding trypticase soy/agar plates, allowed to dry, and incubated at 34°C. At Days 1, 4, and 7 the number of bacterial colonies was counted on each plate. No effort was made to differentiate the type of bacteria.

The experiment was repeated with the dental flosses immersed in the 0.12% CHX solution for 48 hours and 6 weeks to mimic the RCT conditions. In the RCT, subjects would receive their floss, which had been immersed in the solution for at least 48 hours to allow enough time for absorption of the solution into the floss, at baseline and again at Week 6. Since CHX is usually stored in amber-coloured plastic bottles and the floss containers are opaque, white plastic, the CHX within the floss containers was also tested to determine if it was still active after 6 weeks in the container.

A.1.3 Statistical Analyses

2 Sample t-tests and ANOVA with replication were used to compare the test flosses.
A.1.4 Results

The positive control, 0.12% CHX solution, inhibited all bacterial growth, whether it was in the floss container for 48 hours or 6 weeks. Quinine sulfate (QS) did not inhibit bacterial growth (See Table A.1.1), suggesting that this would be a suitable agent for the RCT placebo dental floss.

A.1.4.1 Floss immersed in chlorhexidine for 48 hours

The test flosses, Johnson & Johnson (J&J) waxed floss, Oral-B satin floss (Satin), and Gum unwaxed floss (GUM), which were immersed in CHX for a minimum of 48 hours had no bacterial growth within 24 hours of incubation. (See Table A.1) The J&J, Satin, and GUM test flosses would continue to slow the growth of bacteria over days 4 and 7 compared to their corresponding controls. (See Tables A.2 and A.3) Statistically significant differences were found on Day 4, with the J&J with CHX floss having fewer bacterial colonies than the Satin with CHX floss (p = 0.01), however there was no statistically significant difference between the J&J with CHX floss and the GUM with CHX floss (p = 0.07). After 7 days, there were no statistically significant differences among the Satin, GUM, and J&J flosses with CHX (p = 0.21) The Satin, GUM, and J&J flosses with CHX were significantly better than Oral-B Ultrafloss (Ultrafloss) with CHX (p = 0.01). Ultrafloss with CHX did not inhibit bacterial growth. (See Table A.1)

Eventually, it appeared that the Johnson & Johnson waxed floss would have the fewest number of bacterial colonies than the Satin, GUM, and Ultrafloss test flosses over the 7 days.
Table A.1: Comparison of positive control (0.12% CHX solution), test flosses (immersed in CHX for 48 hours), control flosses, floss with 0.1% quinine sulfate solution (QS) after one day of incubation at 34° C.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHX solution</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J&amp;J floss + CHX</td>
<td>1.00</td>
<td>0</td>
<td>1.73</td>
</tr>
<tr>
<td>Ultrafloss + CHX</td>
<td>501.00</td>
<td>457.00</td>
<td>181.06</td>
</tr>
<tr>
<td>Satin floss + CHX</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GUM floss + CHX</td>
<td>0.67</td>
<td>0.00</td>
<td>1.15</td>
</tr>
<tr>
<td>J&amp;J control floss</td>
<td>318.67</td>
<td>285.00</td>
<td>38.77</td>
</tr>
<tr>
<td>Ultrafloss control</td>
<td>917.67</td>
<td>917.00</td>
<td>7.51</td>
</tr>
<tr>
<td>Satin control floss</td>
<td>320.67</td>
<td>316.00</td>
<td>20.97</td>
</tr>
<tr>
<td>GUM control floss</td>
<td>262.33</td>
<td>260.00</td>
<td>13.62</td>
</tr>
<tr>
<td>Floss + QS</td>
<td>41.67</td>
<td>39.00</td>
<td>15.18</td>
</tr>
</tbody>
</table>

Table A.2: Comparison of positive control (0.12% CHX solution), test flosses (immersed in CHX for 48 hours), control flosses, and floss with 0.1% quinine sulfate (QS) after four days of incubation at 34° C.

<table>
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<th>Day 4</th>
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<td>CHX solution</td>
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<td>0</td>
</tr>
<tr>
<td>J&amp;J floss + CHX</td>
<td>7.33</td>
<td>7.00</td>
<td>1.53</td>
</tr>
<tr>
<td>Ultrafloss + CHX</td>
<td>577.00</td>
<td>592.00</td>
<td>28.99</td>
</tr>
<tr>
<td>Satin floss + CHX</td>
<td>29.00</td>
<td>31.00</td>
<td>10.15</td>
</tr>
<tr>
<td>GUM floss + CHX</td>
<td>4.33</td>
<td>3.00</td>
<td>2.31</td>
</tr>
<tr>
<td>J&amp;J control floss</td>
<td>398.00</td>
<td>362.00</td>
<td>63.24</td>
</tr>
<tr>
<td>Ultrafloss control</td>
<td>904.00</td>
<td>914.00</td>
<td>25.32</td>
</tr>
<tr>
<td>Satin control floss</td>
<td>412.33</td>
<td>406.00</td>
<td>30.48</td>
</tr>
<tr>
<td>GUM control floss</td>
<td>345.00</td>
<td>352.00</td>
<td>19.09</td>
</tr>
<tr>
<td>Floss + QS</td>
<td>232.00</td>
<td>218.00</td>
<td>28.69</td>
</tr>
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</table>
Table A.3: Comparison of positive control (0.12% CHX solution), test flosses (immersed in CHX for 48 hours), control flosses, and floss with 0.1% quinine sulfate (QS) after seven days of incubation at 34° C.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
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<tbody>
<tr>
<td>CHX solution</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J&amp;J floss + CHX</td>
<td>68.67</td>
<td>43</td>
<td>27.18</td>
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<tr>
<td>Ultrafloss + CHX</td>
<td>583.00</td>
<td>592.00</td>
<td>70.29</td>
</tr>
<tr>
<td>Satin floss + CHX</td>
<td>400.67</td>
<td>410.00</td>
<td>59.07</td>
</tr>
<tr>
<td>GUM floss + CHX</td>
<td>293.67</td>
<td>298.00</td>
<td>13.74</td>
</tr>
<tr>
<td>J&amp;J control floss</td>
<td>674.00</td>
<td>707.00</td>
<td>79.39</td>
</tr>
<tr>
<td>Ultrafloss control</td>
<td>963.67</td>
<td>960.00</td>
<td>39.01</td>
</tr>
<tr>
<td>Satin control floss</td>
<td>830.00</td>
<td>818.00</td>
<td>13.01</td>
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<td>GUM control floss</td>
<td>752.33</td>
<td>891.00</td>
<td>150.83</td>
</tr>
<tr>
<td>Floss + QS</td>
<td>737.67</td>
<td>748.00</td>
<td>115.85</td>
</tr>
</tbody>
</table>

A.1.4.2 Flosses immersed in chlorhexidine for 6 weeks

Since the Ultrafloss with CHX had high numbers of bacterial colonies compared to the other CHX soaked dental flosses, it was removed from further testing. The floss with QS also had high numbers of bacterial colonies proving it would be a reasonable placebo for the RCT; hence, it too was also removed from further testing. The J&J, Satin, and GUM flosses with CHX continued to be tested for bacterial growth inhibition. Since the RCT subjects would have the CHX soaked flosses for approximately six weeks before returning the floss to the researchers, further tests were conducted to determine if the flosses soaked in CHX for 6 weeks would have different effects on bacterial growth. Would the prolonged immersion time result in better bacteriostatic effects because the floss absorbed more CHX? Would the CHX soaked floss be less effective because the CHX is not stored in an amber coloured container? When comparing the number of bacterial colonies of the test flosses that had been immersed in the CHX for 48 hours to the number of bacterial colonies of the test flosses immersed in CHX for 6 weeks, there
was no statistically significant difference (Day 1, \( p = 0.20 \); Day 4, \( p = 0.08 \); Day 7, \( p = 0.06 \)). In other words, the amount of time, 48 hours versus 6 weeks, the dental flosses were immersed in the CHX did not affect its bacteriostatic effects. The longer immersion time did not appear to enhance the amount of CHX carried by the floss. Results for the test flosses soaked in CHX for 6 weeks and their corresponding control flosses can be found in Tables A.4, A.5, and A.6. Although the CHX solution in the floss containers continued to inhibit bacterial growth at Day 1, there was some bacterial growth at Days 4 and 7.

Table A.4: Comparison of positive control (0.12% CHX solution), CHX solution from floss containers at week 6, test flosses (immersed in CHX for 6 weeks), and control flosses after one day of incubation at 34°C.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHX control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CHX from JJ floss</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>container</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX from Satin floss</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>container</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX from GUM floss</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>container</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J&amp;J floss + CHX</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Satin + CHX</td>
<td>9.67</td>
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<td>3.06</td>
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<td>GUM floss + CHX</td>
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<td>J&amp;J control floss</td>
<td>70.00</td>
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<td>18.03</td>
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<td>Satin control floss</td>
<td>56.67</td>
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<td>GUM control floss</td>
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</tbody>
</table>
Table A.5: Comparison of positive control (0.12% CHX solution), CHX solution from floss containers at week 6, test flosses (immersed in CHX for 6 weeks), and control flosses after four days of incubation at 34° C.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHX control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CHX from J&amp; J floss container</td>
<td>1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX from Satin floss container</td>
<td>2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX from GUM floss container</td>
<td>3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J&amp;J floss + CHX</td>
<td>7.33</td>
<td>8.00</td>
<td>7.02</td>
</tr>
<tr>
<td>Satin floss + CHX</td>
<td>157.33</td>
<td>163.00</td>
<td>16.26</td>
</tr>
<tr>
<td>GUM floss + CHX</td>
<td>118.33</td>
<td>99.00</td>
<td>44.29</td>
</tr>
<tr>
<td>J&amp;J control floss</td>
<td>322.00</td>
<td>326.00</td>
<td>41.04</td>
</tr>
<tr>
<td>Satin control floss</td>
<td>322.33</td>
<td>290.00</td>
<td>35.89</td>
</tr>
<tr>
<td>GUM control floss</td>
<td>175.33</td>
<td>167.00</td>
<td>8.84</td>
</tr>
</tbody>
</table>

* not done in triplicate; actual count

Table A.6: Comparison of positive control (0.12% CHX solution), CHX solution from floss containers at week 6, test flosses (immersed in CHX for 6 weeks), and control flosses after seven days of incubation at 34° C.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHX control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CHX from J&amp; J floss container</td>
<td>2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX from Satin floss container</td>
<td>3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX from GUM floss container</td>
<td>7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J&amp;J floss + CHX</td>
<td>23.00</td>
<td>13.00</td>
<td>22.72</td>
</tr>
<tr>
<td>Satin floss + CHX</td>
<td>345.00</td>
<td>353.00</td>
<td>69.35</td>
</tr>
<tr>
<td>GUM floss + CHX</td>
<td>267.67</td>
<td>246.00</td>
<td>70.06</td>
</tr>
<tr>
<td>J&amp;J control floss</td>
<td>909.33</td>
<td>909.00</td>
<td>77.08</td>
</tr>
<tr>
<td>Satin control floss</td>
<td>730.00</td>
<td>709.00</td>
<td>103.60</td>
</tr>
<tr>
<td>GUM control floss</td>
<td>958.67</td>
<td>939.00</td>
<td>153.60</td>
</tr>
</tbody>
</table>

* not done in triplicate; actual count
A.1.5 Discussion

There are three possible outcomes for the CHX soaked dental flosses in regards to its effect on the bacteria. One is that the CHX solution did not bind to the dental flosses, but this is unlikely since the CHX soaked flosses had lower numbers of bacterial colonies than their corresponding control flosses. It appears that the CHX was carried in some manner by the flosses to have a bacteriostatic effect. The only exception to this theory is the Ultrafloss®, which had high numbers of bacterial colonies with or without the CHX.

Secondly, the CHX may have been able to bind to the floss, but may not have been released. The Ultrafloss® may have been able to bind a lot of CHX because of its spongy-like texture, but since it did not appear to control the numbers of bacterial colonies, the floss may not have released the CHX. Thirdly, the CHX may have bound to the floss in some manner and was able to be slowly released by the floss to have a bacteriostatic effect. Based on the numbers of bacterial colonies, it appears that the CHX was able to bind to the Johnson & Johnson waxed floss® and be subsequently released to exert a bacteriostatic effect better than the Satin® or GUM® flosses.

The CHX appeared to remain active even though it was stored in a white, opaque plastic container instead of the recommended amber coloured bottle. The bacterial growth on the plates treated with the 0.12% CHX solution from the white plastic containers may have been the result of contamination. The floss containers are not sealed nor are the spools of floss sterilized, so there is the possibility of cross-contamination.
The amount of time that the dental flosses were immersed in the CHX did not appear to improve its bacteriostatic effects. Since the dental flosses were not weighed after being immersed in the CHX solution for 48 hours and 6 weeks, it is unknown if the floss absorbed more CHX over time. The bacterial counts between the test floss immersed in CHX for 48 hours and its counterpart floss immersed in CHX for 6 weeks were not statistically different therefore, it appears that immersion time has no effect on the net results.

Since quinine sulfate did not inhibit bacterial growth, it will be a suitable placebo solution for the RCT. The positive control, 0.12% CHX solution, inhibited all bacterial growth, which is in accordance with the results of other studies.\textsuperscript{4,5}

Evaporation of the CHX solution from the floss containers was a problem. Peridex ® (Zila Pharmaceuticals, Phoenix, Arizona) contains approximately 11% alcohol, which may have contributed to its evaporation. Approximately 50% of the CHX solution evaporated from the floss containers by Week 6. Since the entire spool of floss was not completely immersed in CHX, some areas of the floss may not have carried any CHX. In the RCT, adding more CHX to the floss container between visits would be advisable to ensure that the floss was completely immersed in the CHX solution.
A.1.6 Conclusion

The CHX appears to bind and release from the Johnson & Johnson waxed floss® better than the other flosses that were tested. For the RCT, it is recommended that subjects be given extra CHX solution to top up their floss containers such that the floss spool is completely immersed. Since the dental flosses with the CHX were more effective at inhibiting bacterial growth within one day of having contact with the oral bacteria, the RCT subjects may have better results flossing daily than once every few days.
A.1.7 References


Introduction

You are being invited to take part in this research study because your gums bleed and you are interested in treatments that may stop the bleeding.

Your participation is voluntary

Your participation is entirely voluntary. You may decide whether or not to take part in this study. Before you make your decision, it is important for you to understand what the research study involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study, and the possible benefits, risks, and discomforts.

If you wish to participate in this study, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time without giving any reasons for your decision.

If you do not wish to participate in this study, you do not have to provide any reasons for your decision. Your decision not to participate in the study does not affect your dental hygienist-patient relationship in any way. For example, if you are a patient in the dental practice, you will continue to receive your usual dental care in the usual way.

Please take the time to read the following information carefully. You can discuss it with your family, friends, and dentist before you decide.
Background information about gingivitis and chlorhexidine

Gingivitis is a reversible, gum inflammation caused by the dental plaque on the teeth. Gingivitis is characterized by red, puffy gums, which bleed with brushing or flossing. Untreated, gingivitis may lead to periodontitis. Periodontitis is a serious oral disease, which involves bone destruction and potential tooth loss. The best method for treating gingivitis is to remove the plaque from the teeth by daily toothbrushing and flossing.

Occasionally a dentist or dental hygienist will also recommend chlorhexidine mouthwash in addition to the person’s usual toothbrushing and flossing home care routine to treat gingivitis. Chlorhexidine is an anti-septic mouthwash that has been used to treat gingivitis since the 1950’s. Unfortunately, chlorhexidine mouthwash is unable to reach the areas between the teeth where gingivitis is commonly found. The investigators would like to explore the use of dental floss as a method of applying chlorhexidine to the in-between areas of the teeth to reduce bleeding and plaque, which are associated with gingivitis.

Chlorhexidine mouthwash is approved in Canada for the treatment of gingivitis in humans.

What is the purpose of the study?

The purpose of the study is to determine if flossing with chlorhexidine is more effective than plain dental floss in reducing bleeding and plaque, which are associated with gingivitis.
**Who is sponsoring the study?**

The Canadian Foundation of Dental Hygiene Research and Education and the British Columbia Dental Hygiene Association are sponsoring the study. There is no commercial sponsor for the study. The investigators do not receive any financial gain or benefits from the study.

**Who can participate in the study?**

To be considered for the study, you must meet the following criteria:

1. You have a minimum of 20 natural teeth
2. You have gingivitis (i.e., bleeding gums), not periodontitis (i.e., bone loss)
3. You are able and willing to dental floss daily
4. You are at least 18 years old
5. You are in good health
6. You are a non-smoker

**Who should NOT participate in the study?**

You cannot participate in the study if you have one or more of the following:

1. You require antibiotics before every dental visit
2. You are pregnant or are planning to be pregnant within the next 3 months
3. You are allergic to chlorhexidine or quinine sulfate
4. You have used chlorhexidine, antibiotics or anti-inflammatory drugs within the last 3 months or are currently taking one or all of these medications
5. You are taking Dilantin, Cyclosporin A, Nifedipine or calcium channel blockers, daily aspirin, or anti-coagulants.
6. You have more than 2 crowns and bridges (caps), implants or dentures (false teeth)
7. You have orthodontic appliances (braces)
8. You have periodontitis (bone loss) or gum pockets of 7 mm or greater in more than 2 sites in your mouth

Where is the study taking place?
The study will take place in a dental office at #203- 2031 West 41st Avenue, Vancouver, BC, V6M 1Y7.

How many subjects will be in the study?
There will be a total of 30 volunteers enrolled in this study.

What does the study involve?
This study is double-blinded, which means that neither you nor the investigators will know who is receiving the chlorhexidine floss. However, in case of an emergency, there is a sealed envelope which indicates the type of dental floss you have received. Since the study is double-blinded, a dental floss with placebo solution will also be used. The placebo dental floss will look, smell, and taste like the chlorhexidine dental floss, but will have no active ingredient. The placebo solution is quinine sulfate. Quinine sulfate has no effect on the signs of gingivitis. It is being used because it mimics the taste of chlorhexidine. The placebo is being used to prevent examiner bias. The chlorhexidine or
quinine sulfate solutions, which are in the floss containers, does not promote the growth of germs and are not a source or carrier of infection.

If you decide to participate in this study, the procedures and visits you can expect will include the following.

**Overview of the study**

Screening visit → cleaning visit → Baseline

A 6 wk visit → 12 wk visit

B 6 wk visit → 12 wk visit

**Screening visit**

You will fill in a medical history form. The examiner will look in your mouth to determine if you meet the study’s criteria. If you meet the criteria, you will be asked to sign the consent form. The screening visit takes approximately 30 minutes.

**Cleaning visit**

A dental hygienist will scale and polish your teeth, as well as instruct and assess your flossing technique. The cleaning visit takes approximately 90 minutes.

**Baseline visit**

About 1 week after the cleaning visit, an examiner will look at your teeth and gums. The examiner will disclose your teeth with a disclosing solution, which will stain the plaque on your teeth pink, which can be easily removed by brushing your teeth. The examiner will also measure your gum pockets and determine the number of areas that bleed with a
probe, which is a little ruler. This visit will take approximately 45 minutes. At the end of this visit, you will be assigned to either Group A or B. Group assignments are done randomly so there is an equal chance that you will be enrolled in either group.

**Group A**

If you have been assigned to Group A, you will be asked to dental floss your teeth daily with the chlorhexidine dental floss and record your flossing routine in a flossing diary. You will continue to brush your teeth as usual, but will be asked to refrain from using any other dental cleaning aids or mouthwashes. Professional dental cleanings are also to be avoided during the study period.

**Group B**

If you have been assigned to Group B, you will be asked to dental floss your teeth daily with the placebo dental floss and record your flossing routine in a flossing diary. The placebo dental floss will be similar to the study dental floss, except that it will not have the active ingredient. You will continue to brush your teeth as usual, but will be asked to refrain from using any other dental cleaning aids or mouthwashes. Professional dental cleanings are also to be avoided during the study period.

In both groups, dental floss is considered the standard therapy for the treatment of gingivitis. The dental floss with the chlorhexidine is the test procedure.
Week 6 and Week 12 visits

At each of these visits, an examiner will look at the colour and texture of your gums, assess the amount of plaque on your teeth, determine which areas of your gums bleed, measure your gum pockets, and check your teeth for stain. You will also be asked if you have experienced any side effects. You will be asked to return your dental floss and flossing diary at each visit for a new supply. Each visit will be approximately 45 minutes. Any questions or concerns you have will also be addressed at each of these visits.

Overall duration of the study

This study involves a total of 4 visits, after the initial screening. The overall duration of the study, not including the screening visit, is 3 months. The total number of hours you will be involved in the study is approximately 3½ hours.

What are my responsibilities?

You will be expected to come to all 4 sessions and to floss your teeth daily to remain in the study. If you begin a new drug or become pregnant, please inform the researchers as soon as possible.

What are the possible harms and side effects of participating in this study?

Chlorhexidine has been used in dentistry for 50 years and has been studied extensively in both short and long-term studies. It is safe and low in toxicity. The most common side effect of chlorhexidine is tooth staining, which occurs in three out of four people. The stain, which can be removed with professional polishing, occurs most frequently with the use of 0.2% chlorhexidine mouthwashes. In this study, the chlorhexidine concentration
will only be 0.12%. Since the chlorhexidine will be applied by dental floss to the in-between surfaces of the teeth, tooth staining is expected to be minimal or non-existent.

Six out of 55 subjects experienced a burning sensation in their tongue and one person out of 222 subjects experienced desquamation of the tongue (i.e., the superficial layer of skin on the tongue sloughed off) in other studies. However, this occurred with 0.2% chlorhexidine mouthwashes and is not expected to occur in this study because we are using a lower concentration and the CHX will be confined to the in-between areas of the teeth.

**What are the benefits of participating in this study?**

No one knows whether or not you will benefit from this study. There may or may not be direct benefits to you by taking part in this study. You may or may not see a reduction in the amount of bleeding gums at the conclusion of this study. We hope that the information learned from this study can be used in the future to benefit people with gingivitis.

**What are the alternatives to the study treatment?**

If you choose not to participate in this study, the following treatment options may be available to you:

1. Your usual professional dental care
2. Dental flossing or using other in-between-the-teeth dental aids to clean the in-between areas of your teeth
3. Chlorhexidine mouthwashes
You can discuss these options with your dentist before deciding whether or not to participate in this research project.

What if new information becomes available that may affect my decision to participate?

If new information arises during the research study that may affect your willingness to remain in the study, you will be advised of this information as soon as possible by telephone or e-mail. If a more effective treatment becomes available, it will be offered to you.

What happens if I decide to withdraw my consent to participate?

Your participation in this research study is entirely voluntary. You may withdraw from this study at any time. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future dental care will not be affected.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law this data cannot be destroyed.

What happens if something goes wrong?

Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.
In case of a serious medical event, please report to an emergency room and inform them that you are participating in a clinical study and that the following persons can be contacted for further information: Dr. Donald Brunette at 604-822-2994 or Ms. Pauline Imai at 604-783-9150.

**Can I be asked to leave the study?**

If you are not complying with the requirements of the study or for any other reason, the study investigators may withdraw you from the study. You may be asked to leave the study if you develop one of the study’s exclusion criteria or if unexpected serious adverse events, which affect your safety, become known. If you are removed from the study, you are requested to continue your dental care with your oral health care professional.

The investigators may decide to discontinue the study at any time, or to withdraw you from the study at any time, if they feel that it is in your best interests.

**What happens after the study is finished?**

You may not be able to receive the study treatment after your participation in the study is completed because:

- The treatment may not turn out to be effective
- The treatment may not be commercially available
- Your dental professionals may not feel it is the best option for you
What will the study cost me?

You will have to pay for parking or bus fares to participate in the study. There is no reimbursement for study related expenses.

Will I be offered remuneration for participating in the study?

You will not be paid for participating in this study. However, the professional dental cleaning will be provided at no cost to you (approximate value: $150). You will also have an opportunity to enter a draw for an Oral-B Triumph electric toothbrush (approximate value: $200).

Will my taking part in this study be kept confidential?

Your confidentiality will be respected. All study data will only contain your random subject number. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, for the purpose of monitoring the research, Health Canada and the UBC Research Ethics Board may inspect research and dental records, which identify you, in the presence of the Investigator or his designate. Records, such as the medical history form, which identify you by name, will be stored in the locked files of the Principal Investigator. Only the Principal Investigator and co-investigator will have access to the your information.
Subject Consent to Participate

I have read and understood the subject information and consent form.

I have had sufficient time to consider the information provided and to ask for advice if necessary.

I have had the opportunity to ask questions and have had satisfactory responses to my questions.

I understand that all of the information collected will be kept confidential and the result will only be used for scientific objectives.

I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.

I understand that I am not waiving any of my legal rights as a result of signing this consent form.

I understand that there is no guarantee that this study will provide any benefits to me.

I have read this form and I freely consent to participate in this study.

I have been told that I will receive a dated and signed copy of this form.

I, (print your name) ____________________________, consent to participate in the Flossing with Chlorhexidine Research Study.

Your Signature ____________________________ Date ____________________________

Witness: (Print name) ____________________________

Witness: (Signature) ____________________________ Date ____________________________

Principal Investigator: Dr. Donald Brunette, BSc, MSc, PhD, Professor, Acting Head OBMS, Associate Dean of Research, University of British Columbia

(Signature) ____________________________ Date ____________________________

Co-Investigator: Ms. Pauline Imai, CDA, Dip DH, BDSc, MSc student, University of British Columbia,

(Signature) ____________________________ Date ____________________________
A.3 Medical and Dental History Forms for the Randomized Controlled Trial.

Flossing with Chlorhexidine Research Study
Medical and Dental History Form

Please answer all of the following questions. All information on this form is confidential.

Name: ____________________________
Address: ____________________________ Postal code: ____________________________
Home phone number: ____________________________
Cellular or work phone number at which you can be reached: ____________________________
Date of birth: ____________________________
Male or Female
Physician: ____________________________ Physician’s number: ____________________________
Dentist: ____________________________ Dentist’s number: ____________________________
Emergency contact: name ____________________________ phone number ____________________________

Please check each box and answer each question.
(All information is private and confidential)

YES NO
☐ ☐ 1. Are you under the care of a physician at present? If yes, why?
________________________________________________________________________________________

☐ ☐ 2. Have you ever been a patient in a hospital, undergone any surgery or suffered from any major illness? If yes, indicate year of hospitalization and reason for it.
________________________________________________________________________________________

☐ ☐ 3. Are you taking medicines or non-prescription drugs of any kind? If yes, list the drugs.

Name of drug: ____________________________
Type of drug: ____________________________
Dosage of drug: ____________________________

Name of drug: ____________________________
Type of drug: ____________________________
Dosage of drug: ____________________________

Name of drug: ____________________________
Type of drug: ____________________________
Dosage of drug: ____________________________

Name of drug: ____________________________
Type of drug: ____________________________
Dosage of drug: ____________________________

100
4. Do you have an impairment, such as hearing/seeing? If yes, what is it?

☐ ☐

5. Have you ever taken cortisone or steroid medication? If yes, when was the last time it was taken?

☐ ☐

6. Have you any allergies? If yes, name the substance or drugs to which you are allergic.

☐ ☐

7. Are you allergic to latex or latex products?

☐ ☐

8. Have you ever had a peculiar reaction to anaesthetics, medicines, or injections (e.g., local anaesthetics, penicillin)? If yes, what was the occasion and describe what happened.

☐ ☐

9. Do you have or have you had any of the following diseases or problems?

☐ ☐ (a) Heart trouble, heart attack or stroke

☐ ☐ (b) Rheumatic fever or heart murmur

☐ ☐ (c) Difficulty breathing

☐ ☐ (d) Chest pain

☐ ☐ (e) High blood pressure

☐ ☐ (f) Diabetes, if yes, present treatment

☐ ☐ (g) Fits, seizures, convulsions, or epilepsy

☐ ☐ (h) Kidney disease
YES  NO

(i) Infectious or communicable diseases, if yes, identify 

(j) STD (sexually transmitted diseases), if yes, identify 

(k) Yellow jaundice or liver disease

(l) Indigestion, heartburn, ulcer

(m) Endocrine disorder, e.g., Thyroid

(n) Nervous disorder

(o) Bone, muscle or joint disorder, e.g., Arthritis

(p) Cancer

(q) Radiotherapy

(r) Artificial joints or valves

(s) History of Hepatitis A , B , C (pick check those that apply)

(t) Immune deficiency, e.g., AIDS

(u) H.I.V. positive

(v) Are there diseases or medical problems that run in your family? If yes, identify 

(w) Do you bleed excessively?

(x) Do you faint easily?

(y) Do you smoke? If yes, how much?

(z) Do you drink tea and/or coffee? If yes, how much?
FEMALES ONLY:

☐ ☐ Are you pregnant? If yes, what is your due date? ________________________

DENTAL HISTORY

YES NO

☐ ☐ 1. Have you visited a dentist within the last year?

☐ ☐ 2. Do you brush your teeth? If yes, how often do you brush?

_________ times a day

☐ ☐ 3. Do you floss your teeth? If yes, how often do you floss?

_________ times a day/week/month (please circle one)

☐ ☐ 4. Are you currently bleaching or whitening your teeth?

☐ ☐ 5. Are you currently using chlorhexidine mouthwash?

____________________________________  ______________________________
(Your signature)                        (Date)

Thank you for completing this medical/dental history form.
A.4 Data Collection Form for the Randomized Controlled Trial.

Flossing with Chlorhexidine Research Study: Data Collection

Floss Box # Date: Initials of examiner: 

Ging Index (GI)
0 = Absence of inflam
1 = SI colour change only
2 = Mod glazing, redness, edema, hypertrophy, BOP
3 = Marked redness, hypertrophy, tend spontaneous bleeding, ulcer

Stain Index (SI)
0 = No visible stain
1 = Light, barely visible stain
2 = Mod stain
3 = Hvy stain

Plaque Index (PI)
0 = No plaque
1 = Film of plaque
2 = Moderate
3 = Heavy

Pocket Depth (PD)
Ging margin to base of pocket, in mm.

Bleeding on Probing (BOP)
+ = present
- = absent
A.5 Subject Flossing Diary for Randomized Controlled Trial

Flossing Diary

Please enter the day and time that you flossed your teeth. If you miss a day, please write the date and enter “missed” in the time column.

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Date</th>
<th>Time</th>
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<tbody>
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<table>
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<th>Week 2</th>
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<th>Date</th>
<th>Time</th>
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Next page...
You are scheduled for the Week 6 assessment appointment on

(week)  (day)  (time)  (location)

** Please bring the dental floss and the flossing diary with you.**

If you need to change your appointment, please call Pauline at 604-783-9150.
Please give 48 hours notice. It is important that you have your assessment period within a few days of the original scheduled appointment.
Flossing Diary (continued)

Please enter the day and time that you flossed your teeth. If you miss a day, please write the date and enter "missed" in the time column.

<table>
<thead>
<tr>
<th>Week 7</th>
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<td>Date</td>
<td>Time</td>
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<tr>
<th>Week 8</th>
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Week 12

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You are scheduled for the Week 12 assessment and cleaning appointment on

\[\text{(day)} \quad \text{(time)}\]

at

\[\text{(location)}\]

** Please bring the dental floss and the flossing diary with you.**

If you need to change your appointment, please call Pauline at 604-783-9150.

Please give 48 hours notice. It is important that you have your assessment period within a few days of the original scheduled appointment.
How to floss your teeth

1. Cut off about 18 inches of floss and wind most of it around one of your middle fingers. Wind the remaining floss around the same finger of the opposite hand. This finger will take up the floss as it becomes dirty. Hold the floss tightly between your thumbs and forefingers. Sometimes it helps to make an “L” shape with your index finger and thumb, then pinch the floss between the index finger and thumb.

2. Guide the floss between your teeth using a gentle “sawing” or back and forth motion. Never snap the floss into the gums.

3. When the floss reaches the gum line, curve it into a C shape against one tooth. Gently slide it into the space between the gum and the tooth.

4. Hold the floss tightly against the tooth. Gently rub the side of the tooth, moving the floss away from the gum with up and down motions.

5. Repeat this method on the rest of your teeth.

6. Don’t forget the back side of your last tooth.

There is a video clip on dental flossing technique on the website: http://flossing.dentistry.ubc.ca
A.6 The University of British Columbia Clinical Research Ethics Board Study Approval Certificates

Attached as follows:

A.3.1 Certificate of Expedited Approval #C05-0513, November 8, 2005
A.3.2 Certificate of Expedited Approval: Amendment #C05-0513, April 12, 2006
A.3.3 Certificate of Expedited Approval: Amendment #C05-0513, July 17, 2006
A.3.4 Certificate of Expedited Approval: Renewal #H05-70513, October 6, 2007
A.3.5 Certificate of Expedited Approval: Amendment #H05-70513, November 23, 2007
# ETHICS CERTIFICATE OF EXPEDITED APPROVAL: AMENDMENT

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<tr>
<th>PRINCIPAL INVESTIGATOR:</th>
<th>DEPARTMENT:</th>
<th>UBC CREB NUMBER:</th>
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<tr>
<td>Donald M. Brunette</td>
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## INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

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Other locations where the research will be conducted:
Private dental office: Dr. Ian Low, #203-2031 W. 41st Ave. Vancouver, BC, V6M 1Y7

## CO-INVESTIGATOR(S):

- Pauline H. Imai

## SPONSORING AGENCIES:

- Canadian Foundation for Dental Hygiene Research & Education - "Flossing with chlorhexidine research study"
- Unfunded Research - "Flossing with Chlorhexidine Research Study"

## PROJECT TITLE:

Flossing with Chlorhexidine Research Study

REMINDER: The current UBC CREB approval for this study expires: October 10, 2007

## AMENDMENT(S):

- N/A

## AMENDMENT APPROVAL DATE:

- November 23, 2006

## CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the University of British Columbia Clinical Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approval of the Clinical Research Ethics Board by one of: