Comparison of the Effects of Different Mouthrinses on Intraoral Bacteria in Orthodontic Patients: A Randomized Clinical Trial

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ABSTRACT

Introduction: Increased plaque and enamel decalcification around fixed orthodontic appliances are common problems in orthodontics. The objective of this randomized clinical trial was to compare the amount of oral bacteria found in plaque around orthodontic brackets in 3 groups of orthodontic patients; one using a mouthrinse containing fluoride, xylitol and a pH neutralizing agent with a group using a standard over-the-counter fluoride mouthrinse and a control group that was not provided a rinse.

Methods: Fifty-four adolescent patients undergoing comprehensive orthodontic treatment were randomly assigned to 3 groups; Group 1 was given a mouthrinse containing fluoride, xylitol and a pH neutralizing agent (CariFree®, Oral Biotech, Albany, Oregon), Group 2 was given an over-the-counter fluoride mouthrinse (ACT®, Chattem Inc., Chattanooga, TN) and Group 3 was not provided with a mouthrinse. Plaque specimens were collected from the maxillary canines and assayed for oral bacteria using ATP-bioluminescence at pretreatment (T0), 6 weeks (T1) and 15 weeks (T2). The results were compared for significant differences. Post-study surveys were given to study subjects to assess oral hygiene home care and compliance. Analysis of intra- and inter-group bacterial counts were made using 2-way ANOVA with Tukey post-hoc comparisons. Pearson Correlation Coefficient and T-tests were used to analyze the survey data.

Results: Comparing bacterial counts within groups, Group 1 showed no significant changes between T0 to T1 and T1 to T2. Groups 2 and 3 showed significant increases between T0 and T1 ($p<0.05$), but no change T1 to T2. Comparison among groups showed no significant differences at T0, T1 or T2. There was no significant difference in compliance between groups. In Groups 1 and 2, there was no correlation with compliance and taste of the mouthrinses.

Conclusions: Results suggest that use of a mouthrinse containing fluoride, xylitol, and a pH neutralizing agent during fixed appliance orthodontic treatment helps prevent significant increases in oral bacterial counts after 6 and 15 weeks of treatment. However, no significant differences were found when comparing groups using an over-the-counter fluoride mouthrinse or when no mouthrinse was provided.

INTRODUCTION

One of the side effects of fixed orthodontic appliance therapy is the development of demineralization or white-spot lesions (WSL) in tooth enamel around the base of the brackets bonded to the teeth. Studies show that compared to individuals with no history of orthodontic treatment, orthodontic patients tend to accumulate higher amounts of plaque and show an increase in caries-causing bacteria that can lead to increased WSL formation.[1-10] Gorelick et. al.[3] showed that up to 50% of orthodontic patients may experience decalcification of at least one tooth, while Enaia et. al.[11] found a 60.9% incidence of WSL during multibracket appliance treatment. Previous studies have also shown that there are significant differences in the frequency of white spot lesions among individual teeth where the most affected teeth are the maxillary lateral incisors followed by the maxillary and mandibular canines.[3,12,13] The least affected teeth are the maxillary first molars.[3]

The two main groups of bacteria responsible for tooth demineralization are *mutans streptococci* and *lactobacilli*.[14,15] These bacteria produce lactic acid that rapidly lowers the pH in plaque and diffuses into the underlying enamel producing decalcification.[16] Orthodontic appliances have been found to augment the presence of *S. mutans* and *lactobacilli*, both of which are among the first bacteria to colonize around brackets.[1,2,7]

Many different approaches have been proposed to decrease intraoral bacteria and prevent enamel decalcification in orthodontic patients. Proposed strategies are similar to those used for general caries prevention, such as patient motivation,[17] nutritional counseling,[18] plaque staining,[18] professional tooth cleaning,[19] fluoridation,[20,21] chlorhexidine rinses,[22] and xylitol gum.[23] Other

methods include application of casein phosphopeptidesamorphous calcium phosphate (CPP-ACP)[24] and argon-laser enamel surface attenuation.[25] These methods can be supplemented with specially designed orthodontic materials including fluoride-releasing bonding agents[26] and sealants[27] and fluoride-releasing elastomeric modules and ligature ties.[28] There are numerous studies that demonstrate the effect of various mouthrinses on intraoral bacteria, plaque accumulation, gingivitis and other periodontal indices in non-orthodontic patients. In orthodontic patients, there have been several studies published on the effect of different mouthrinses on intraoral bacteria and the development of white-spot lesions.[29-34]

The method of ATP bioluminescence for rapid assessment of bacterial contamination has been used extensively in the food industry[35-37] as well as with medical devices[37] and hospitals,[38] personal care products,[39] and pharmaceutical manufacturing.[40] More recently, ATP bioluminescence has been introduced in the dental field for the assessment of intraoral bacteria, oral hygiene, and caries risk.[41] This method is based on the reaction between bacterial ATP and the enzyme luciferase with the cofactor luciferin. Hydrolysis of ATP by luciferase emits yellow-green light that can be detected by a luminometer and reported as a relative light unit (RLU). The RLU values reported using ATP-driven bioluminescence have been shown to be highly predictive of the total numbers of bacteria, total streptococci, and mutans streptococci in plaque and saliva.[41,42] The application of ATP-driven bioluminescence is a potentially useful tool in the rapid, chair-side quantification of bacterial load and in the assessment of oral hygiene during orthodontic treatment.[41,42]

In this current randomized clinical trial, the amount of oral bacteria found in plaque around orthodontic brackets was assessed and compared among three groups of patients: Group 1 was

provided a mouthrinse containing fluoride, xylitol and a pH neutralizing agent, Group 2 was provided an over-the-counter (OTC) fluoride mouthrinse, and Group 3 was a control group not provided with any mouthrinse. Bacterial counts were evaluated before orthodontic treatment and at 6 and 15 weeks after orthodontic treatment was begun using ATP-driven bioluminescence.

METHODS AND MATERIALS

***Test Subjects*:** The patients considered for participation in this study presented for treatment at the orthodontic clinic of the Oregon Health & Science University (OHSU), Portland, OR. Patients underwent an initial screening exam to determine eligibility to participate in the study according to the following criteria: must be between the ages of 10-18 years, have fully erupted maxillary canines, and assigned for full comprehensive orthodontic treatment. Each patient's consent for inclusion in the study was obtained at the treatment consultation appointment. As they were enrolled, each patient was randomly assigned to one of three groups using a randomization table stratified by gender. Left- or right-handedness was also recorded for each participant. On the day orthodontic brackets were bonded, all patients were given standardized oral hygiene instructions and mouthrinses were given to those patients in the mouthrinse groups.

Of the 142 patients screened for enrollment, 5 declined to participate in the study, 46 did not meet the inclusion criteria, and the remaining 91 patients were randomly assigned to one of the three study groups. Thirty-eight patients were dropped from the clinical trial at 15 weeks either because the maxillary canines had not been bonded with orthodontic brackets during the study period, the patients failed to show for their appointments, or plaque readings were not taken at the proper appointments. A CONSORT flow chart of participants through each stage of the trial is shown in Figure 1 and sex and age distribution is shown in Table I.

IRB and Human Subjects Consent: This study was reviewed and approved by the OHSU Institutional Review Board (IRB). In addition to the consent form for routine orthodontic care currently in use in the OHSU Orthodontic Clinic, the caregiver of each subject was given a

second consent form specifically for the clinical study. Other than the mouthrinse protocol for each group, the study called for no additional treatment or procedures other than those normally performed during initial bonding, orthodontic adjustment visits, or as instructed for home oral hygiene procedures.

Description of Experimental Groups: Patients in Group 1 received a mouthrinse containing fluoride, xylitol, and a pH neutralizing agent (CariFree®, Oral Biotech, Albany, OR) to be used with home care during orthodontic treatment. According to the manufacturer's instructions, the rinse was to be applied in two phases: a treatment phase and a maintenance phase. The active ingredients in the treatment rinse are xylitol, sodium hydroxide, sodium hypochlorite, and a pH neutralizing agent. The treatment rinse was to be swished vigorously for 1 minute, twice daily for 1 month. The maintenance rinse contains sodium fluoride 0.05%, xylitol, and a pH neutralizing agent and was to be was to be swished vigorously for 1 minute, twice daily for two months, then once daily for the remainder of treatment . Group 2 patients were given an over-the-counter mouthrinse (ACT®, Chattem Inc., Chattanooga, TN) containing 0.05% sodium fluoride and was to be was to be swished vigorously for 1 minute, once daily. Patients in Group 3 were not provided with any mouthrinse but did receive standard oral hygiene instructions. Patients in this group were not asked to eliminate the use of a mouthrinse, if they currently used one, nor were they encouraged to use a specific mouthrinse during treatment.

Plaque Collection and ATP-Driven Bioluminescence of Specimens: Plaque samples were collected from patients before orthodontic treatment (T0) and at an average of 6 weeks (T1) and 15 weeks (T2) into treatment. At T0, plaque was collected by rubbing special swabs that include an upper reservoir containing proprietary luciferin, luciferase, and extraction components

(CariScreen Swabs, Oral Biotech) across the facial surface of each maxillary canine. The swabs were then placed in a swab holder and the upper reservoir was broken, draining the components over the swab. This was then mixed in the swab holder for 1 minute. The swap holder was then placed into the hand-held ATP-Bioluminescence Meter (CariScreen Caries Susceptibility Testing Meter, Oral Biotech) and the Relative Light Units (RLU) were measured and recorded (Fig. 2). (Samples at T0 were taken by various orthodontic residents in the orthodontic clinic)

At T1 and T2, the archwire was removed and plaque was collected from the maxillary canines using the 4-pass technique as described by Pellegrini et. al.[42] where a scaler was passed along the four edges of the bracket base. The plaque was then diluted in 10 ml of sterile saline solution and vortex-mixed for one minute. After mixing, 1 ml of the solution was removed using a calibrated pipet and placed in a CariScreen Swab holder (Oral Biotech) containing the bioluminescence reagents. After mixing for one minute, the swab holder was placed in the hand-held ATP Bioluminescence meter for RLU measurements. For each maxillary right and left canine, four RLU measurements were made from the plaque solutions and averaged for each side. To account for the 10-fold dilution, the RLU readings were multiplied by 10. At T1 and T2, various orthodontic residents removed the plaque around the orthodontic brackets, but the principle investigator did all of the mixing and RLU measurements. The reason for the 10-fold dilution was to account for the fact the CariScreen meter was not sufficiently sensitive to RLU readings above 7,000 which occurred in patients with heavy plaque accumulations.

Surveys: At the completion of the study, surveys were given to all participants in the study. These surveys were developed to ascertain information on each patient's compliance with use of the mouthrinse, tooth brushing behavior and rating of mouthrinse taste. The questions included

"How many days a week did you use the mouthrinse", "When did you use the rinse", "How many times a day do you brush your teeth", "On average, how much time before your orthodontic appointment did you brush your teeth", and "Do you think your teeth were cleaner with the mouthrinse than without it". Compliance and taste of the mouthrinses were evaluated using a visual analogue scale (VAS). Since group 3 did not receive a mouthrinse, they were given a different survey that did not contain the questions related to the mouthrinses, but were asked if they used a mouthrinse at home during the study period and, if so, how often the mouthrinse was used.

Statistical Analysis: Of the 91 patients enrolled, 79 had plaque samples collected at T0, 54 patients had samples collected at T1 (15 in Group 1, 20 in Group 2, 19 in Group 3) and 41 patients at T2 (11 in Group 1, 14 in Group 2, 16 in Group 3; see Table II). Descriptive statistics, including mean values for bacterial counts at T0, T1, and T2 from ATP-driven bioluminescence determinations (in RLUs) and corresponding standard deviations were calculated. Differences in the mean bacterial counts from ATP-driven bioluminescence determinations (in RLUs) at the various time points within and among the 3 groups were tested using 2-way ANOVA with Tukey post-hoc comparison (SAS version 9.1, SAS Institute, Cary, NC) where $P < 0.05$ was considered statistically significant.

Pearson Correlation Coefficient was used to analyze correlations between survey date results within each group. T-test was used to compare compliance and mouthrinse taste between Groups 1 and 2.

RESULTS

Statistical analysis using ANOVA showed no significant differences between the RLU values measure from the right maxillary canine versus the left maxillary canine, therefore, the values from the right and left side were averaged to get a mean RLU value at each time point between groups. There were also no significant differences between the values obtained from the right and left maxillary canines and the handedness of the patients.

Mean RLU values recorded at T0, T1, and T2 are shown in Figure 3. When assessing the change in RLU value between T0 and T1 within groups, there was no significant change in Group1, whereas there were statistically significant increases in Groups 2 (P=0.048) and 3 (P=0.005). Between T1 and T2 there was no significant change within any of the groups (Table III). When comparing for inter-group differences, no significant differences were found for the absolute RLU values measured at T0, T1 or T2 (Table III). Similarly, no significant inter-group differences were found among groups comparing RLU changes from T0 and T1 and from T1 and T2 (Table IV).

Surveys: Forty-eight of the 54 patients in the study responded to the surveys (88.9%). Mean values for compliance, number of days the mouthrinse was used, number of times the patients brushed their teeth, and taste rating from the surveys are shown in Table V. Although they were not asked to use a mouthrinse, 60% of patients in group 3 did use a mouthrinse on their own volition during treatment for an average of 4.6 days per week.

Within Groups 1 and 2, Pearson Correlation Coefficients showed there was a positive correlation with compliance and the number of days per week the rinse was used. There was no correlation

with compliance and taste of the mouthrinses. In Group 3, there was a positive correlation with number of days a mouthrinse was used with the number of times a day a subject brushed their teeth.

T-test results comparing compliance and taste of the mouthrinses between Groups 1 and 2 showed no significant difference in compliance between groups. However, there was a significant difference in taste between mouthrinses with patients in Group 1 rating the mouthrinses (treatment and maintenance rinses) significantly lower ($P<0.01$) than Group 2.

With regard to the question of when patients brushed their teeth before orthodontic appointments, 76.9% of patients in Group 1, 73.7% in Group 2, and 86.7% in Group 3 reported brushing their teeth within 2 hours of their orthodontic appointment.

DISCUSSION

This is the first study to compare the effect of a mouthrinse containing fluoride, xylitol, and pH neutralizing agent with a fluoride-only mouthrinse on intraoral bacteria in orthodontic patients. Our findings show that in 2 of the 3 groups (Groups 2 and 3) there was an increase in bacterial counts after the placement of fixed orthodontic appliances. These results are consistent with previous studies showing the presence of orthodontic appliances increases plaque accumulation and cariogenic bacteria.[1,5,8] In contrast, Group 1 (CariFree® mouthrinses) did not show a statistically significant increase in intraoral bacterial count after orthodontic appliances were placed, indicating a possible benefit to the use of a mouthrinse that contains xylitol, fluoride, and a pH neutralizing agent. This result is also consistent with a previous study demonstrating that regularly chewing xylitol gum reduces plaque around orthodontic appliances and can reduce the risk of caries in orthodontic patients.[23] Xylitol promotes mineralization by increasing salivary flow, is non-fermentable by oral bacteria, and is known to inhibit growth and metabolism of mutans streptococci.[43]

Even though Group 1 was the only group not to show a significant increase in bacteria after 6 and 15 weeks of treatment, when the changes from T0 to T1 and T1 to T2 were compared among all the groups, there were no significant differences. This finding could be due to a number of factors. During the design of this study, it would have been unethical to restrict the control group (Group 3) from using a mouthrinse or any of the groups from brushing before orthodontic appointments. Even though patients in Group 3 were not encouraged to use a mouthrinse, according to the survey results, 60% of the patients in Group 3 reported that they used a mouthrinse at least 3 times a week. This may have reduced the possibility of finding a potential

difference in the change in bacterial count between the mouthrinse groups and the control group. The finding of a high percentage of patients using a mouthrinse in the "no-rinse" control group could have been due in part to an increased awareness of mouthrinse use. During the informed consent process, patients were made aware that they were participating in a mouthrinse study and that they would possibly be provided with a mouthrinse for use during their orthodontic treatment. If the patient was assigned to the control group, they were perhaps more aware of the potential importance of the use of a mouthrinse and decided to use one even though a mouthrinse was not provided. Interestingly, patients in Group 3 that brushed their teeth more frequently also were more likely to use a mouthrinse, indicating that a heightened awareness of oral hygiene correlates with mouthrinse usage. Another limiting factor that may have narrowed differences among the groups could be related to the finding that at least 73% of the patients indicated that they had brushed their teeth within 2 hours of their orthodontic appointment when the collection of the plaque samples would have taken place. Brushing activity could have masked the effect of the mouthrinses due to a disruption of the plaque.

There is some evidence that a daily fluoride mouthrinse will reduce the severity of WSLs if used during treatment with fixed braces.[44] Ogaard et. al.[30] compared patients using a sodium fluoride mouthrinse with patients not using a rinse and found significantly decreased lesion depths in patients using the mouthrinse. Even though the current study did not measure effects on WSL formation, we found that the use of a fluoride mouthrinse did not significantly influence the amount of bacteria around orthodontic brackets when compared to a group that was not provided a mouthrinse. This may suggest that fluoride's effect on reducing WSL formation, as shown in previous studies, may relate more to reduced demineralization of enamel than on reduced

number of bacteria.[45,46] In contrast to our findings related to the over-the-counter fluoride rinse, two studies using mouthrinses containing 0.2% chlorhexidine gluconate[32] or octenidine dihydrochloride[33] have shown to decrease intraoral bacteria in orthodontic patients. However, comparisons to these studies are limited as both of the previous studies assessed the rinses over a shorter time period and neither study had a "no-rinse" control group. The patients in the chlorhexidine gluconate study served as their own controls where bacteria samples were taken before and then after using the rinse.[42] From these studies, it is unknown if there is added benefit of a 0.2% chlorhexidine gluconate rinse over a fluoride mouthrinse. Tufekci et. al.[34] showed that the use of Listerine® mouthrinse containing essential oils can reduce the amount of plaque and gingivitis in orthodontic patients but bacteria counts and WSL formation were not measured. In the current investigation, comparison of within-group longitudinal results suggest that xylitol and/or the pH neutralizing agent may be determining factors that increase the efficacy of the mouthrinse against the buildup of bacterial plaque.

Previous studies show that teeth most susceptible to WSL formation are the maxillary lateral incisors.[3,12] Orthodontic brackets on maxillary lateral incisors are often placed near the gingival margin in order to obtain proper vertical incisal edge relationship with the neighboring central incisor, thus, in the current study this positioning would have made it difficult for plaque removal between the bracket and gingival margin without causing bleeding. If blood was introduced into the plaque samples, the ATP luminometer would have also measured the ATP produced from cells within the blood. Since this study aimed to measure the ATP produced only by intraoral bacteria, the maxillary canines were used for collection of plaque samples since brackets are generally placed further away from the gingiva in these teeth. Maxillary canines

have been shown to have the second highest incidence of white spot lesions in the maxillary arch.[13] However, this created the need to screen more patients as many adolescent patients seeking orthodontic treatment did not yet have fully erupted maxillary canines.

There are numerous studies that indicate a difference in plaque accumulation within the mouth based on the person's handedness. In general, right handed individuals have increased plaque accumulation on the buccal surfaces of teeth on the right side.[47-50] This study, however, found that right handed individuals had no difference in the amount of bacteria surrounding the maxillary right canine bracket as they did with the maxillary left canine bracket. Interestingly, information on plaque in left handed individuals is sparse but Addy et. al.[50] did note that left handers do not have a favored side and brush equally well, or badly, on the left and right buccal surfaces. This was consistent with findings in this study, however, unlike the previous study that had 100 left handed individuals, this study had 7, a sample too limited for drawing definitive conclusions.

The self-contained swab collection system and hand-held luminometer used in this study has been shown to provide rapid and reliable bacterial measurements at chair-side for oral hygiene assessment of children.[41] In the current study we found the system to have limited range. The optimal range for the hand-held luminometer is anywhere from 1000-7000 RLUs. For this reason, dilution of the plaque samples was necessary after braces were placed because the RLU values would have been over 7000 if undiluted. There were times in the study, however, when the readings were below the optimal range of the meter when the samples were diluted, but would have been too high if they were undiluted. This may have contributed to inaccurate

measurements for some of the readings. To minimize the potential inaccuracies and avoid dilutions, a luminometer with a larger range would have been more ideal.

Because the standard deviations for the RLU values were so high and there was no significant differences between groups in the intra-oral bacteria count change from T0 to T1, a statistical power analysis was done after the study period was complete. The power analysis showed that 975 patients would be required in each group, or 2,925 total patients, to investigate a significant difference between Groups 1 and 2 at 0.8 power. To investigate a significant difference between Groups 1 and 3 at 0.8 power, 174 patients in each group or 522 patients in total would be required. It would be a challenge in almost any setting to enroll this many patients.

According to the surveys, the ACT® mouthrinse had a significantly better taste than the CariFree® mouthrinses. However, this did not affect the patient's compliance, as the reported compliance between the groups was similar. This could be interpreted to mean that taste of a mouthrinse does not affect the compliance with mouthrinse use, or possibly that the subjects in the CariFree® group (Group 1) continued to use the mouthrinse, despite the taste, because they felt required to do so as study subjects. We are unaware of any studies correlating mouthrinse compliance with taste.

In the current study, the principle investigator had to enlist the help of orthodontic residents in screening patients for study entry, obtaining plaque samples and distributing surveys. Although residents were given daily reminders of the study protocol, due to the busy clinic schedule, many patients who could have been included in the study were lost, some plaque samples were missed and a few surveys were not distributed.

Even though the current study did not show any significant differences in intraoral bacteria counts between the two mouthrinse groups or compared to the group where no mouthrinse was provided, the within-group finding that bacteria counts did not significantly increase with patients using the mouthrinse containing xylitol and pH-neutralizing agent suggest that these may be useful factors in mouthrinses. Due to the conflicting intra- vs. inter-group differences, the current study does not provide conclusive evidence regarding the potential of patients to develop WSLs using the different mouthrinses.

CONCLUSIONS

1) Longitudinal results comparing changes in bacteria counts within mouthrinse groups suggest that use of a mouthrinse containing fluoride, xylitol and a pH neutralizing agent may help prevent significant increases in oral bacterial counts after placing fixed orthodontic appliances.
2) Comparing groups at 6 and 15 weeks of treatment, there was no difference in bacteria counts associated with use of a mouthrinse containing fluoride, xylitol, and a pH-neutralizing agent, relative to an over-the-counter fluoride mouthrinse, or when no mouthrinse was prescribed.
3) Mouthrinse taste did not affect the patient's compliance in using the mouthrinse.

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FIGURE LEGENDS

Figure 1. CONSORT flow chart of study participants through each stage of the trial (n, number of patients)

Figure 2. Cariscreen® (Oral BioTech) Caries Susceptibility Testing Meter and Swabs.

Figure 3. Mean RLU values measured with ATP-driven Bioluminescence from plaque samples taken from the Maxillary Right and Left Canines over time periods T0, T1, and T2.

Figure 1.

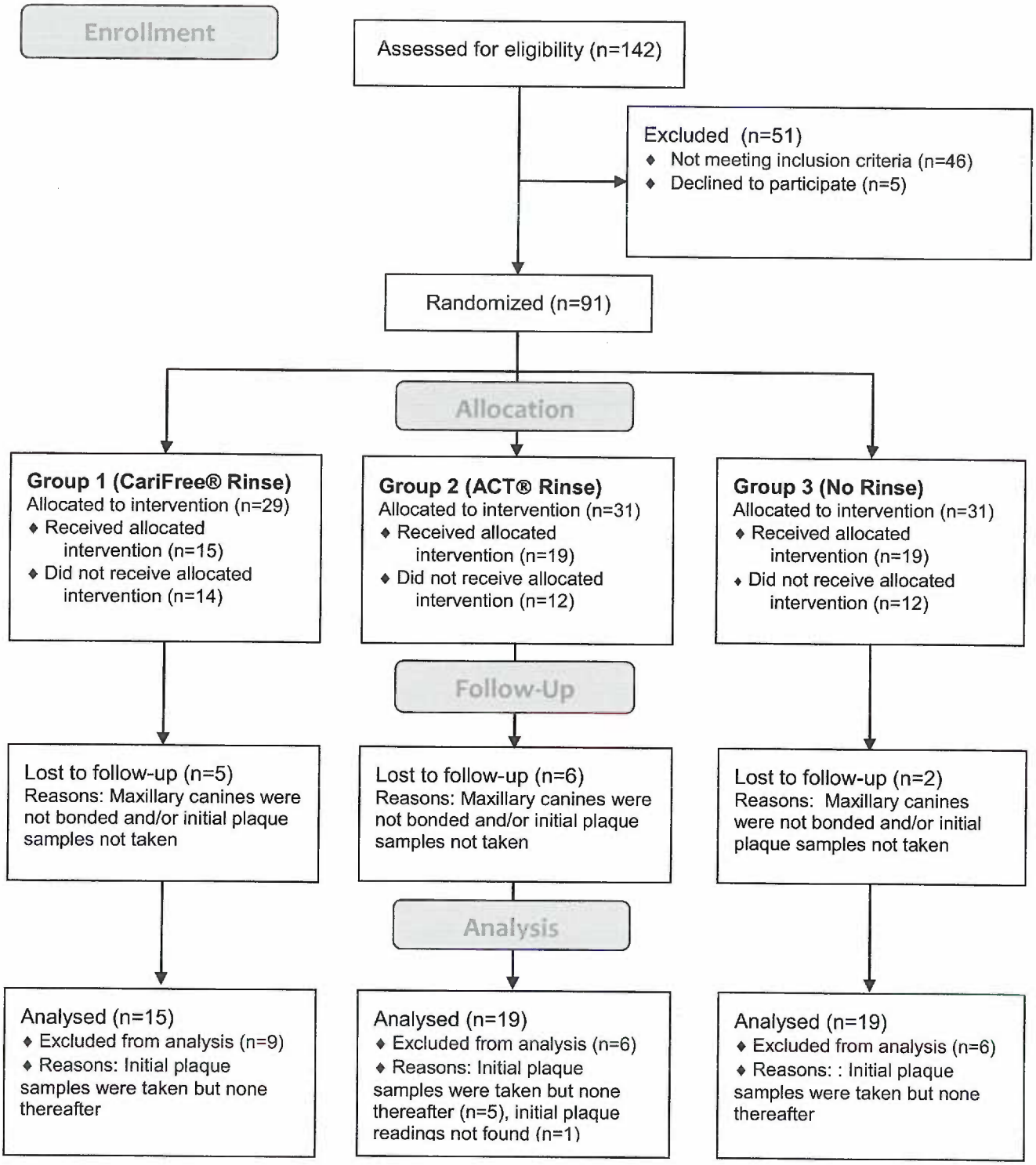
Enrollment
Assessed for eligibility (n=142)
Excluded (n=51)
♦ Not meeting inclusion criteria (n=46)
♦ Declined to participate (n=5)
Randomized (n=91)
Allocation
Group 1 (CariFree® Rinse)
Allocated to intervention (n=29)
♦ Received allocated intervention (n=15)
♦ Did not receive allocated intervention (n=14)
Group 2 (ACT® Rinse)
Allocated to intervention (n=31)
♦ Received allocated intervention (n=19)
♦ Did not receive allocated intervention (n=12)
Group 3 (No Rinse)
Allocated to intervention (n=31)
♦ Received allocated intervention (n=19)
♦ Did not receive allocated intervention (n=12)
Follow-Up
Lost to follow-up (n=5)
Reasons: Maxillary canines were not bonded and/or initial plaque samples not taken
Lost to follow-up (n=6)
Reasons: Maxillary canines were not bonded and/or initial plaque samples not taken
Lost to follow-up (n=2)
Reasons: Maxillary canines were not bonded and/or initial plaque samples not taken
Analysis
Analysed (n=15)
♦ Excluded from analysis (n=9)
♦ Reasons: Initial plaque samples were taken but none thereafter
Analysed (n=19)
♦ Excluded from analysis (n=6)
♦ Reasons: Initial plaque samples were taken but none thereafter (n=5), initial plaque readings not found (n=1)
Analysed (n=19)
♦ Excluded from analysis (n=6)
♦ Reasons: : Initial plaque samples were taken but none thereafter

Figure 2.

Figure 3.

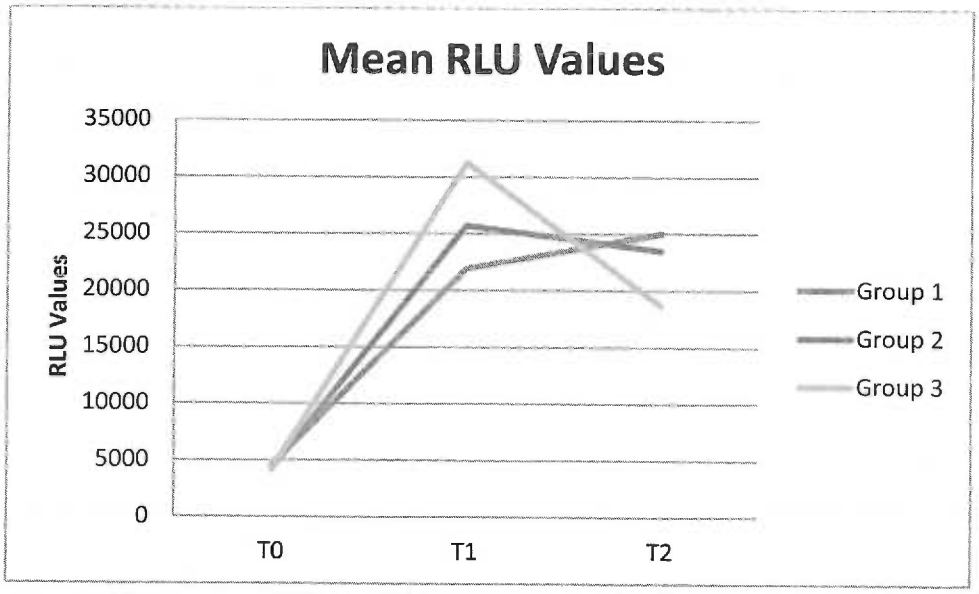
Mean RLU Values
RLU Values
35000
30000
25000
20000
15000
10000
5000
0
T0
T1
T2
Group 1
Group 2
Group 3

Table I. Sample Distribution by Sex and Age

	Group 1	*Group 2*	*Group 3*	*Total*
Subjects (n)	15	20	19	54
Male	4	6	8	18
Female	11	14	11	36
Mean age (y)	14.61	14.25	14.02	

Table II. Study Enrollment

	# of Subjects Entered Into Study	*# of Subjects Started*	*# of Subjects with 6 Week Values*	*# of Subjects with 15 Week Values*
Group 1	29	24	15	11
Group 2	32	26	20	14
Group 3	31	29	19	16
Total	91	79	54	41

Table III. Mean RLU Values and Standard Deviations at T0, T1, and T2

Time	Group 1 (CariFree®)	Group 2 (ACT®)	Group 3 (No Rinse)	Significance Between Groups
0	4550 (3275)	4194 (3047)	4142 (3012)	NSD
1	21900 (23160)	24870 (23640)†	25520 (27880)†	NSD
2	25010 (21790)	23430 (27600)	18670 (21970)	NSD

Significant within groups when compared with the previous time-point, † $P < 0.05$

Table IV. Change in Mean RLU Values Between T0-T1 and T1-2

	Δ T0-T1	***P* Value**	**Δ T1-T2**	***P* Value**
Group 1 (CariFree®)	17350	0.4007	3716	1
Group 2 (ACT®)	20670	0.0486	1034	1
Group 3 (No Rinse)	25520	0.0053	11359	0.8641
Significance Between Groups	NSD		NSD	

Table V. Means of Survey Data Results

	Compliance*	Days Rinse Used/Week	Brushing/Day	Taste*†
Group 1	7	5.5	2.4	4.4††
Group 2	7.5	5.1	2.1	8.3
Group 3	NA	2.8	2.5	NA

* Measured on a Visual Analogue Scale from 0-10

† Significant difference between groups ($P<0.01$)

†† Average of taste values for Treatment Rinse and Maintenance Rinse

LITERATURE REVIEW

Caries Formation

The process of caries formation has been studied extensively. Plaque that accumulates on the surface of teeth contains bacteria that produce acids as products of their metabolism of carbohydrates.[1] These acids, mainly lactic acid, can dissolve the carbonate-rich regions of the hydroxyapatite crystal structure of tooth enamel or dentin.[2-4] This process is known as demineralization. The first clinical sign of demineralization is the "white spot" lesion (WSL). This is a small area of subsurface demineralization beneath the dental plaque.[5] This lesion can progress to caries if carbohydrates are ingested and remineralization does not occur.

White spot lesions, or decalcification, of labial and buccal enamel around bracket pads in orthodontically treated patients can pose a serious problem during orthodontic treatment. Studies show that orthodontic patients tend to accumulate higher amounts of plaque and show an increase in caries-causing bacteria which can lead to increased WSL formation.[6-15] Gorelick et. al.[8] showed that up to 50% of orthodontic patients may experience decalcification on at least one tooth. A more recent article by Enaia et. al.[16] showed a 60.9% incidence of WSL during multibracket appliance treatment. There are also significant differences in the frequency of white spot lesions among individual teeth. The most affected teeth are the maxillary lateral incisors followed by the maxillary and mandibular canines.[8,17] The least affected teeth are the maxillary first molars.[8]

The two main groups of bacteria that are responsible for tooth demineralization are the mutans streptococci and the lactobacilli.[1] These bacteria produce lactic acid which rapidly lowers the

pH in the plaque and diffuses into the underlying enamel.[3] Featherstone[5] states that these two groups of bacteria, either separately or together, are the primary causative agents of dental caries. Metal orthodontic brackets have been found to augment the presence of *S. mutans* and they are among the first bacteria to colonize around brackets.[6,12]

ATP Bioluminescence

ATP is present in all living cells, including bacteria. The ATP molecule consists of a pentose sugar (ribose) with a purine base (adenine) and three phosphate groups. Energy is released through the hydrolysis of these phosphate groups and is used for various biological reactions in cells.

ATP bioluminescence for rapid assessment of bacterial contamination is based on a reaction that occurs naturally in the firefly.[18] The enzyme Luciferase, in the presence of D-luciferin, oxygen, and magnesium ions, utilizes the energy from ATP to oxidise D-luciferin to produce light.[19] The intensity of emitted light can be measured by a luminometer and is reported as a relative light unit (RLU).[20] The intensity of the light measured by the luminometer is directly proportional to the amount of ATP in the sample and therefore correlates with cellular contamination.[21] Crouch et. al.[22] showed a linear relationship between cell number and measured luminescence using the luciferin-luciferase reaction and Griffiths et. al.[23] stated that the amount of light emitted during the reaction is proportional to the number of bacteria from which the ATP was released.

The method of ATP bioluminescence for rapid assessment of bacterial contamination has been used extensively in the food industry[18,22] as well as with medical devices[24] and hospitals,[25] personal care products,[26] and pharmaceutical manufacturing.[21] More recently, ATP bioluminescence has been introduced in the dental field for assessment of intraoral bacteria, oral hygiene, and caries risk.[27] ATP-driven bioluminescence has been shown to be highly predictive of the total numbers of bacteria, total streptococci, and mutans streptococci in plaque and saliva.[27-29] Using ATP-driven bioluminescence can serve as a useful tool in the rapid, chair-side quantification of bacterial load and in the assessment of oral hygiene during orthodontic treatment.[27,29]

The ATP bioluminescence Cariscreen® test (Oral BioTech, Albany, OR) is a semi-quantitative means of assessing caries susceptibility in the dental office. In the Cariscreen® test, a swab is used to collect plaque from the surface of teeth. The swab is then immediately bathed in a solution that causes lysis of the plaque bacteria and the ATP is measured. This method has several advantages over the more time-consuming standard selective plating assays, including; faster results, ease of use, and possible chair-side application.

Prevention of WSLs

Many different procedures and remedies have been proposed to remedy the decalcification problem. Many proposed strategies are similar to those used for general caries prevention, such as patient motivation,[30] nutritional counseling,[30] plaque staining,[31] professional tooth cleaning,[32] fluoridation,[33,34] chlorhexidine,[35] and xylitol gum.[36] These methods have also been

supplemented with specific materials that orthodontists can use, including; fluoride-releasing bonding agents[37] and sealants,[38] fluoride-releasing elastomeric modules and ligature ties,[39] and argon-laser enamel surface attenuation.[40]

Probably the most important way to help prevent WSL in orthodontic patients is through patient education, counseling, and motivation. Geiger et. al.[41] showed a significant correlation between poor patient compliance with home care preventive procedures and the formation of WSL. They showed no statistically significant differences between the compliance levels of males and females or among different age groups. They also stated that professional oral hygiene instruction and regular professional cleaning has been shown to be effective in reducing decalcification, especially when the degree of compliance with the recommended home care preventive protocol is poor.[41]

Fluoride can be incorporated into the crystal lattice of dental enamel by the replacement of hydroxy groups or by the redeposition of dissolved hydroxyapatite as less soluble fluoridated forms, such as fluorapatite or fluorhydroxyapatite. This results in a structure that is more resistant to acid dissolution.[4] During orthodontic treatment, fluoride can be applied to the teeth in various ways, including topical (fluoridated toothpaste, mouthrinse, gel and varnish) and adhesive methods (fluoride-releasing cements and elastomeric modules and chains).[9]

A systematic review done in 2005 by Benson et. al.[42] sought to evaluate the different modes of fluoride delivery and their effect on WSL in orthodontic patients. They concluded that there is "some evidence that a daily sodium fluoride mouthrinse will reduce the severity of demineralization associated with orthodontic appliances and that glass ionomer cement used for

bonding reduces the incidence and severity of WSL compared with a composite resin." However, they found little evidence as to which method or combination of methods to deliver fluoride is the most effective. They recommend using a 0.05% sodium fluoride mouthrinse in addition to a fluoride toothpaste. This is based on research carried out in non-orthodontic patients. There are also a few studies that show using toothpaste with high concentrations of fluoride reduce WSL in orthodontic patients.[43,44] However, there have been other clinical trials showing contrasting results with respect to fluoride. Blinkhorn et.al[45] did a three-year longitudinal clinical study investigating the combined effects of the daily use of 7600ppm fluoride toothpaste and 500ppm sodium fluoride mouthrinse. They found that the combined use of these products did not produce any additional benefits to the use of either product alone and suggested that a daily use of a fluoridated mouthrinse could be omitted from the orthodontic home care program.

Fluoride varnish is another manner of applying topical fluoride to the enamel of teeth. Ogaard et al.[46] showed that regular application of fluoride varnish reduces WSL formation on bracketed maxillary incisor teeth. Fluoride varnish, however, needs to be reapplied frequently because it is removed by toothbrushing and oral function within just a few days.[47]

Fluoride-releasing bonding agents were developed in the 1980s in an effort to achieve constant topical fluoride exposure without relying on patient compliance.[9] The first of these fluoride-releasing bonding agents were glass ionomer cements. Since then, other cements have been developed such as resin-modified glass ionomers and fluoride-containing composites (compomer) cements. Most of these fluoride-releasing bonding cements have similar fluoride-releasing characteristics[9] with an initial "burst" of fluoride release during the first few days after

bonding, which then declines to a lower but more stable rate.[48] Gorton et. al.[49] showed that fluoride-releasing glass ionomer cement for bonding orthodontic brackets successfully inhibits caries in vivo. "This cariostatic effect was localized to the area around the brackets and was statistically significant after 4 weeks."[49] Marcusson et. al.[50] showed an average 16.5% decrease in WSL formation when using glass ionomer cement versus composite resin cement. Fluoride-releasing composite resin cements have been shown to release significantly smaller concentrations of fluoride over time when compared with glass ionomer and resin modified glass ionomer cements.[51] However, there is a significant decrease in shear and tensile bond strengths of glass ionomer cements when compared to composite resin.[52]

Another proposed method of delivering topical fluoride in orthodontic patients is the advent of fluoride-releasing elastomeric modules and chains. In *in vitro* studies, these elastomeric products release fluoride with an initial burst during the first 24 to 48 hours, but then decreases in a logarithmic pattern over time.[53] Storie et. al.[53] also found that fluoride-releasing elastomeric chain had a significant loss of delivery force and "was unable to deliver a force within the optimal range for tooth movement after one week." An *in vivo* study done in 2007 by Miura et. al.[54] showed no significant differences in the number of *Streptococcus mutans* in saliva or plaque in the area surrounding the fluoride-releasing or conventional elastomeric ligature ties. Needless to say, these fluoride-releasing elastomeric modules have not gained much popularity.

Using an argon laser has been suggested in the prevention of enamel decalcification.[55,56] It is thought that the mechanism of action is the creation of microspaces that stabilize ions during an acid attack rather than allowing them to be lost from the enamel.[57] The available calcium, phosphate and fluoride ions in saliva are then precipitated into these microspaces, increasing the

resistance of the enamel to demineralization and increasing the uptake of minerals.[57] It's use in orthodontic treatment has had mixed results. Anderson et. al.[58] tested the in vivo effects of argon laser irradiation on enamel decalcification after 5 weeks around orthodontic bands. They found that the average lesion depth in the laser group was reduced by 94.1% and the average lesion area was reduced by 94.4% when compared with the control group. However, Elaut et. al.[59] found that after 14 months of treatment, there was no significant difference in decalcification and plaque accumulation when using an argon laser for curing around orthodontic brackets.

Chlorhexidine has been widely used in dentistry because of its ability to inhibit plaque formation and reduce bacteria in the oral cavity.[60] There are various ways to apply chlorhexidine including gels, sprays, varnishes, mouthwashes, chips, chewing gums, and dentifrices.[61] Most studies determining the effect of chlorhexidine also used fluoride in conjunction with the chlorhexidine. Most of these studies found no significant differences in WSL formation when chlorhexidine was used[62-64] but did find a significant decrease in numbers of mutans streptococci.[64,65] One study that did not use fluoride in conjunction with chlorhexidine did show, however, a significant decrease in the number of new carious lesions after debonding.[65]

Xylitol is a sugar substitute that is completely non-fermentable by oral bacteria.[66] It has been shown that regular xylitol consumption decreases mutans streptococci counts, decreases the amount of plaque, reduces caries formation, and even decreases the mother-child transmission of mutans streptococci resulting in primary prevention of caries in young children.[67-70] The same bacterial and plaque results also occur in orthodontic patients that consume xylitol.[71] However, there are no studies on the consumption of xylitol during fixed orthodontic appliance treatment and the appearance of WSL.

The application of casein phosphopeptidesamorphous calcium phosphate (CPP-ACP) may help to prevent enamel demineralization, however, there isn't sufficient clinical trial evidence regarding its long-term effectiveness.[72] CPPACP incorporates nanocomplexes into dental plaque and onto tooth surfaces, acting as a calcium and phosphate reservoir.[9] Studies show that CPP-ACP incorporated into dental plaque significantly increase the levels of plaque calcium and phosphate ions.[73,74,75] When intraoral pH lowers, calcium and phosphate ions are released to produce a supersaturated concentration of ions in the saliva, which then precipitates calcium-phosphate onto the exposed tooth surface.[76] This limits enamel demineralization and enhances remineralization.[73,75] An in-vitro study reported that enamel lesions remineralized with topical exposure to CPP-ACP gum and were more resistant to subsequent acid challenges compared with normal remineralized enamel.[77] CPP-ACP is available in several forms; gum, solution, MI Paste and MI Paste Plus (GC America, Alsip, IL).

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APPENDIX 1

Comparison of Mouthrinses on Intra-oral Bacteria in Orthodontic Patients

Survey

Group 1

Thank you for participating in the study on the effect of mouthrinses in orthodontic treatment. As part of the study, we would like you to complete this brief survey. We ask you to be completely honest in this survey. The results of this survey will NOT be seen by your orthodontist and will in no way affect your treatment. Your name is not placed on the survey, only a unique code identifier.

For questions 1 and 4, there is a line with a scale going from 0-10. For those questions please place an "X" on the line where you determine is the best answer.

Example: On a scale of 0 to 10, with 0 being "terrible" and 10 being "excellent", please indicate on the line how you are feeling today?

If you have any questions with the survey, let your orthodontist know and he/she will get the head researcher for the study to help clarify. When you are done with the survey, please fold it in half and give it to your orthodontist. The orthodontists are instructed to not look at the surveys.

Thank you once again for participating in the study. You can continue to request mouthrinses if you run out. It is through research and volunteers like you that help us deliver the best treatment to our patients and continually improve our methods of treatment.

Patient # _____ Date: _____

1. On a scale of 0 to 10, with 0 being "never" and 10 being "completely followed instructions", please indicate on the line where you think your use of the mouthrinse would be?

0 10

   a. How many days a week did you use the mouthrinse? _____

   b. When did you use the rinse? *Circle best answer.* Morning Night Both Other

      i. If *other*, please specify time of day. _____

2. How many times a day do you brush your teeth? _____

3. On average, how much time before your orthodontic appointment did you brush your teeth? *Circle the best answer.*

<1 hour 1-2 hours 2-4 hours 4-6 hours 6-8 hours >8 hours

4. On a scale of 0 to 10, with 0 being "worst taste ever" and 10 being "really pleasant taste", please indicate on the line what you think about the taste of your mouthrinse. *Only rate the mouthrinses that you used.*

   a. CariFree Treatment rinse? 

   b. CariFree Maintenance rinse? 

5. Do you think your teeth were cleaner with the mouthrinse than without it? Yes No

Comparison of Mouthrinses on Intra-oral Bacteria in Orthodontic Patients

Survey

Group 2

Thank you for participating in the study on the effect of mouthrinses in orthodontic treatment. As part of the study, we would like you to complete this brief survey. We ask you to be completely honest in this survey. The results of this survey will NOT be seen by your orthodontist and will in no way affect your treatment. Your name is not placed on the survey, only a unique code identifier.

For questions 1 and 4, there is a line with a scale going from 0-10. For those questions please place an "X" on the line where you determine is the best answer.

Example: On a scale of 0 to 10, with 0 being "terrible" and 10 being "excellent", please indicate on the line how you are feeling today?

If you have any questions with the survey, let your orthodontist know and he/she will get the head researcher for the study to help clarify. When you are done with the survey, please fold it in half and give it to your orthodontist. The orthodontists are instructed to not look at the surveys.

Thank you once again for participating in the study. You can continue to request mouthrinses if you run out. It is through research and volunteers like you that help us deliver the best treatment to our patients and continually improve our methods of treatment.

Patient # _____ Date: _____

1. On a scale of 0 to 10, with 0 being "never" and 10 being "completely followed instructions", please indicate on the line where you think your use of the mouthrinse would be?

0 10

   a. How many days a week did you use the mouthrinse? _____

   b. When did you use the rinse? *Circle best answer.* Morning Night Both Other

      i. If *other*, please specify time of day. _____

2. How many times a day do you brush your teeth? _____

3. On average, how much time before your orthodontic appointment did you brush your teeth? *Circle the best answer.*

<1 hour 1-2 hours 2-4 hours 4-6 hours 6-8 hours >8 hours

4. On a scale of 0 to 10, with 0 being "worst taste ever" and 10 being "really pleasant taste", please indicate on the line what you think about the taste of your mouthrinse.

   a. ACT Mouthrinse?

0 10

5. Do you think your teeth were cleaner with the mouthrinse than without it? Yes No

Comparison of Mouthrinses on Intra-oral Bacteria in Orthodontic Patients

Survey

Group 3

Thank you for participating in the study on the effect of mouthrinses in orthodontic treatment. As part of the study, we would like you to complete this brief survey. We ask you to be completely honest in this survey. The results of this survey will NOT be seen by your orthodontist and will in no way affect your treatment. Your name is not placed on the survey, only a unique code identifier.

If you have any questions with the survey, let your orthodontist know and he/she will get the head researcher for the study to help clarify. When you are done with the survey, please fold it in half and give it to your orthodontist. The orthodontists are instructed to not look at the surveys.

Thank you once again for participating in the study. It is through research and volunteers like you that help us deliver the best treatment to our patients and continually improve our methods of treatment.

Patient # _____ Date: _____

1. How many days a week do you use a mouthrinse? _____

   a. What mouthrinse do you use? _____

   b. When did you use the rinse? *Circle best answer.* Morning Night Both Other

      i. If *other*, please specify time of day. _____

2. How many times a day do you brush your teeth? _____

3. On average, how much time before your orthodontic appointment did you brush your teeth? *Circle the best answer.*

   <1 hour 1-2 hours 2-4 hours 4-6 hours 6-8 hours >8 hours

APPENDIX 2

FUTURE RESEARCH

The use of mouthrinses to help prevent WSLs is promising. However, there are numerous other questions that can be answered with future research. The following is a list of future research opportunities:

1) In the current study, initial photos of the maxillary canines were taken before orthodontic appliances were placed. Patient's in the study could be followed throughout the remainder of treatment and WSL formation could be evaluated comparing the initial photos with final photos. Would the mouthrinse containing fluoride, xylitol, and pH neutralizing agent prevent WSL formation better than the over-the counter fluoride mouthrinse?
2) The current study had a no-rinse control group. But as stated in the thesis, many of these patients used a mouthrinse anyway. Another study could use the same protocol as the current study but instead of a no-rinse group, include a placebo mouthrinse group. This could answer the question of whether the act of swishing a liquid with no active incredients in the mouth disrupts the bacteria sufficiently to reduce the bacteria around orthodontic appliances.
3) An experiment isolating xylitol and the pH neutralizing agent could be proposed to determine which ingredient is the greater factor in reducing intraoral bacteria.