Title
Effect of Xylitol-Wipe on Mutans Streptococci Virulence

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Effect of Xylitol-Wipe on Mutans Streptococci Virulence

by

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THESIS

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Abstract

Title: Effect of Xylitol-Wipe on Mutans Streptococci Virulence

Purpose: In a randomized clinical trial, we found a significant reduction of new caries in young children with daily use of xylitol-wipes over one year without reduction on mutans streptococci (MS) levels. The purpose of this study was to determine whether the use of xylitol-wipes for one year was due to selection of xylitol-resistant MS, altered the rate of acid production of MS and/or altered biofilm formation ability of MS.

Methods: Unique MS genotypes were isolated from the xylitol-wipe group and the placebo-wipe group at baseline and at 1 year. These isolates were examined for their xylitol sensitivity, rate of acid production and ability to form biofilm. Xylitol sensitivity was determined by comparing bacterial growth curves in TPY glucose broth with or without the addition of 1% xylitol. Acid production was monitored by the means of pH drop for 30 minutes after each isolate was grown in the presence of glucose. Sucrose dependent biofilm formation of each genotype was quantified using crystal violet assay.

Results: At one year, there was no significant difference in prevalence of xylitol-resistant MS genotypes between the xylitol-wipe and the placebo-wipe groups. There was significant decrease difference in plateau pH within each group from baseline to 1 year ($p<0.05$). The change in acid profile in the xylitol-wipe group from baseline to 1 year was similar to that observed in the caries vs. no caries group. There were no statistical significant differences in biofilm formation of MS isolated from the two wipe groups at baseline or one year ($p>0.05$).

Conclusions: This prospective randomized clinic trial has shown no correlation between caries reduction and selection of xylitol-resistant MS. Furthermore, xylitol-wipe use did not alter the ability of MS to form biofilm. However, there is evidence to suggest that after one year xylitol wipe use would select MS with decrease acid production ability. Future studies are needed to identify other possible mechanisms of caries prevention resulting from the use of xylitol-wipes in children.

1. Introduction

Dental caries remains as one of the most prevalent chronic infectious diseases in children. For the past decades, dental caries prevention has been a main focus in dentistry. The well-recognized group of cariogenic bacteria in humans are Mutans streptococci (MS), namely *streptococcus mutans* and *streptococcus sobrinus.* Increased levels of MS have been associated with early childhood caries[1]. Two main virulence factors related to the cariogenic ability of MS are its high affinity of adherence to tooth surfaces in the presence of sucrose as well as its ability to produce acid by fermenting various dietary sugars leading to dental caries formation[2]. To reduce dental caries, researchers have been seeking for effective regimens to either reduce MS levels in the oral cavity or its virulence factors. Xylitol has shown promising results clinically. The mechanism of xylitol in preventing dental caries however are currently not well understood. A better understanding at the molecular level will aid in proper dosing and use of xylitol.

2. Literature Review

2.1 Clinical Studies

Xylitol, a five-carbon noncariogenic natural sugar alcohol found in many fruits and vegetables[3, 4] has been shown to be a possible candidate in combating MS. Xylitol's influences on MS have peaked many scientists' interest since 1980s. In a double-blind randomized controlled trial, Holgerson et al.[5] investigated the effect of xylitol-gum use for 4 weeks on plaque formation and MS levels compared to sorbitol and maltitol gum

group in schoolchildren (grades 1-6). The results showed that after 4 weeks, visible dental plaque was significantly reduced in both groups indicating that chewing gum alone provides a cleansing effect. In addition, MS levels in saliva were significantly reduced in the xylitol group but no significant reduction was found in the sorbitol and maltitol gum group. These results suggest that xylitol has a possible advantage over sorbitol/maltitol in dental caries reduction. This study along with others with different xylitol regimen[6-8], highlight that after short-term use (weeks to a few months) of xylitol results in reduction of MS levels.

Makinen et al.[9] studied the effect of xylitol gum or xylitol-sorbitol gum for 24 months on MS in 8-9 years old students with a 39-month follow-up and a no-gum control group. At 12 months, MS levels were decreased in all groups with no statistical differences. The authors proposed that this decrease reflected the effects of improved oral hygiene and diet habits as a result of oral hygiene instructions given to all students at the beginning of the study. At 24 months, the levels of MS in plaque as well as saliva continued to decrease significantly in both xylitol group and xylitol-sorbitol group. Interestingly at 39 months, only the xylitol group showed continual decrease in the levels of MS. These results suggest that xylitol not only decreases the level of MS during its use but also has a possible long-term effect after cessation of its use. Contrary to this study, Soderling et al investigated maternal use of xylitol gum for 21 months, and showed no reduction of MS levels in the mothers[10]. Subsequent studies also showed that reduction of MS level are not sustained after long-term use of xylitol (>12 months)[11, 12].

Despite the inconsistent effects of xylitol use on levels of MS colonization,

prevailing evidence has shown that success of maternal use of xylitol gum can prevent both MS transmission and dental caries in children[10, 13, 14]. A recent study by Milgrom et al also demonstrated successful reduction of dental caries by direct use of xylitol syrup in infants[15].

2.2 Mechanism of Xylitol in Caries Prevention

Although clinical evidence strongly suggests the effectiveness of xylitol in caries prevention, its mechanism is not fully understood. Few studies have been conducted to investigate the biological mechanism of xylitol on MS. A number of studies have suggested that xylitol may affect several virulence factors of MS closely related to its cariogenic properties. These studies, as well as studies related to the formation of xylitol resistant MS strains, are further described below.

2.2.1 Xylitol Resistant MS

Xylitol is a non-cariogenic sugar alcohol, and therefore cannot be utilized by MS. This may explain why the MS proliferation is initially inhibited by xylitol use. Xylitol competes with glucose to be transferred into the cells of MS through the PTS pathway[16]. Once inside the cell, xylitol is metabolized by MS to produce xylitol-5-phosphate[17]. Xylitol-5-phosphate cannot be further metabolized and accumulates in the cell. Its accumulation leads to inhibition of MS cell glycolysis and glucose uptake and eventually results in inhibition of MS growth. Xylitol-5-phosphate accumulated in the bacterial cells can be dephosphorylated and expelled creating a "futile" cycle for MS[3]. This cycle uses up energy and hence decreases bacterial growth and acid production even further.

Trahan[3] hypothesized that the inhibition of MS growth by xylitol contributes to the reduction of MS levels after xylitol consumption.

However, long-term xylitol consumption leads to the emergence and selection of xylitol-resistant MS [16, 18, 19, 20]. Xylitol resistant mutants are defined as strains that are incapable of accumulating toxic xylitol phosphate and their growth are not inhibited by xylitol. It is possible that xylitol resistance may explain the different effects of xylitol in short term as compared to long-term xylitol consumption.

2.2.2 Acid Production

Recently, Kakuta et al.[21] demonstrated that xylitol not only hampered the growth, but also reduced acid production of MS in the presence of other dietary sugars (glucose, galactose, maltose, lactose and sucrose). This suggests that xylitol can be effective when consumed in the presence of other dietary sugars in our normal diets. Several other studies also verified reduced acid production in dental plaque after long term xylitol consumption[22-25]. The decrease in acid production potentially explains the reduction in dental caries after xylitol use. To date, no study has systemically investigated acid production of MS isolates in subjects treated by xylitol when compared to MS isolates from the baseline or control groups. It is still unclear if caries prevention effect of xylitol is related to the decrease in MS growth, inhibition of acid production of MS, or the emergence of less virulent xylitol-resistant MS strains.

2.2.3 Biofilm Formation

Adhesion to tooth surface, especially in presence of sucrose, is another

characteristic of MS that could influence virulence[26]. There are two mechanisms in which MS adheres to tooth surfaces: sucrose-independent and sucrose-dependent adhesion. Sucrose-independent adhesion is responsible for the initial colonization of MS to the tooth pellicle by the means of adhesin-like cell surface molecules[27]. In the presence of sucrose, the enzyme glucosyltransferase synthesizes glucan, especially water-insoluble glucan, which is responsible for sucrose-dependent adhesion and plaque formation of MS[2]. Water-insoluble glucan not only aids in adhesion of MS but also acts as an extracellular matrix that changes the physico-chemical properties of dental plaque to make it more cariogenic[26].

The impact of xylitol on sucrose dependent adhesion of MS is controversial. Wunder et al.[28] reported that xylitol did not inhibit the activity of isolated glucotransferase *in vitro*. Assev et al.[29] did not show differences in glucan formation between xylitol-sensitive and xylitol-resistant strains. However, the study by Lee et al.[20] showed that MS isolates from women who had consumed xylitol gum for 12-months formed smaller, less undulated, and smoother colonies when compared to the control group. In addition, they found that gtfB gene (glucosyltransferase-I) expression also decreased in the xylitol group.

From these studies, many researchers have hypothesized that the success of maternal use of xylitol in prevention of MS transmission and dental caries in infants is due to its ability to select xylitol resistant MS strains that are easily shed in saliva. However, no study has been performed to confirm this hypothesis.

2.3 Significance

Xylitol has been shown in many studies to be noncariogenic and also to have protective effects against tooth decay in children[4]. Maternal use of xylitol has also been shown to be a successful avenue to prevent MS transmission and dental caries in infants. However, the biological mechanism of xylitol in caries prevention, and its effects on MS remains unclear. In this study, we have for the first time, analyzed the effects of xylitol on MS selection and retention, and determined how xylitol alters the biological properties of MS, including biofilm formation and acid production in young children.

2.4 Aims and Hypothesis

The specific aims of this study are to investigate whether 1-year use of xylitol wipes in children will 1) select for xylitol resistant MS strains, 2) select MS with reduce acid production and/or 3) select MS strains with less sucrose-dependent biofilm formation using MS isolated from our previous xylitol-wipe study. The study will provide us with the fundamental understanding on the mechanisms of xylitol on MS and caries prevention.

3. Materials and Method

3.1 General Study Design

The MS genotypes used in this current study were isolated from our previous study of the effectiveness of xylitol wipes in caries prevention[30]. The study was approved by The Committee on Human Research of the University of California San Francisco,

approval number 10-04899. Mother-child pairs that fulfilled the inclusion criteria were recruited for the study until 44 pairs were reached. All mothers had at least 1 active carious lesion within the past year and all children were 6-35 months old. The children were randomly assigned into either the xylitol-wipe or placebo-wipe groups. Mothers were instructed to use the two wipes 3 times daily for their infants in addition to routine oral hygiene care. The number of decayed, missing, or filled primary teeth (dmfs) were determined through examinations of the child at baseline and 1 year. Saliva samples were collected at baseline and 1 year for MS enumeration. Ten MS colonies were isolated and genotyped by arbitrarily primed polymerase chain reaction from each saliva sample. The MS genotypes were stored in TSB glycerol broth at -80^{o}C. Only subjects that completed the study and had MS infection were included in this study. These MS genotypes used for the current study are summarized in Table 1.

Table 1: MS Genotypes

	Subjects with MS		**MS Genotypes**	
	Xylitol (n=18)	**Placebo (n=11)**	**Xylitol**	**Placebo**
Baseline	4	4	11	6
1 year	10	7	16	19

3.2 Xylitol Sensitivity Assay

A xylitol sensitivity assay was performed by comparing the growth of MS in Brain Heart Infusion broth (BHI) with or without xylitol as previously described by Trahan et al[18]. MS isolates stored in TSB glycerol broth at -80^{o}C were inoculated in 2ml of Brain Heart Infusion broth (BHI) with 1% glucose and grown overnight. Two-tenths of the

overnight broth was re-inoculated in 2ml of BHI with 1% glucose and grown overnight. Approximately one-third of the cells (OD_{490} = 0.02) in the exponential growth phase were then added to 6mL TPY supplemented with 0.2% (11mmol/L) glucose. The medium were mixed on a Vortex and divided in two. Thirty micro-liters of 50% xylitol (final concentration 1%, 66mmol/L) were added to one half and 30μL of sterile water were added to the other. Growth was monitored every 2 hours for 10 hours and a final reading at 24 hours with the optical density at 490 nm using the Precision Microplate Reader (Molecular Devices, USA). A strain was considered xylitol-resistant when its growth curve on glucose in the presence of xylitol was identical to that in its absence. For the xylitol-sensitive strain, its growth curve on glucose in the presence of xylitol was suppressed when compared to its absence. All assays were run in triplicates and repeated once.

3.3 Rate of Acid Production

Acid production assay of MS was tested using the methods described by Kakuta et al[21]. The recovered MS genotypes (see above) were inoculated in 2 ml of 1% glucose BHI broth and grown anaerobically overnight at 37^{o}C. After overnight growth, 1 ml of the broth was again inoculated in 10ml of BHI with 1% glucose and grown overnight. This was to ensure a homogenous mixture was obtained for each MS sample. To test for acid production, the MS cells were then suspended by centrifugation (7000g for 15 minutes) at the logarithmic phase of growth, washed three times with a phosphate buffer solution [2 mM potassium phosphate buffer (PBS) containing 150 mM KCl and 5 mM $MgCl_2$] and suspended in the PBS. Three-tenth ml of bacterial cell suspension (OD = 1.5

at 540 nm) was transferred to a water bath for 4 minutes at 35˚C. The initial pH was measured using a Thermo Scientific Orion 2-Star Benchtop pH Meter (Thermo Fisher Scientific, USA). Subsequently, 30μL of glucose was added to the suspension to allow for acid production. The pH change was monitored and recorded every minute by the Thermo Scientific Orion 2-Star Benchtop pH meter for 30 minutes. The acid product constant K, plateau pH and half-time for acid production was calculated for each assay using Prism 7 software for Mac. The half-time ln(2)/K is defined as the time it takes for the pH to drop to half of the difference between initial and plateau pH where time is dependent on K, the rate of acid production.

3.4 Sucrose-Dependent Biofilm Formation

Sucrose-dependent biofilm formation by the MS genotypes was measured by crystal violet staining, modified from the biofilm assay used by Yoshida and Kuramitsu[31]. The MS genotypes were inoculated in 2 ml of 1% glucose BHI broth and grown overnight. Two-tenths of the overnight broth was re-inoculated in 2ml of BHI with 1% glucose and grown overnight. One-tenth ml BHI of the overnight bacterial broth was inoculated into a 12 x 75mm round-bottom polystyrene tube (BD Biosciences, USA) containing 1 ml of BHI 0.5% sucrose broth and incubated for 48 hours. The broth and the non-adherent bacteria were poured off and the bacterial biofilm attached to the walls of the tube were washed with 4 ml volumes of distilled water. This washing process was repeated three times. The biofilm was stained for 2-5 minutes with 2 ml of 0.2% crystal violet. The crystal violet solution was poured off and the biofilm was washed with distilled water until no visually detectable color was evident in the wash water. The tubes

were inverted and air-dried overnight at room temperature. One ml of ethanol containing 3% hydrochloric acid was used to extract the crystal violet bonded to the biofilm for 48 hours. Aliquots (150 µl), in triplicate, from each tube were transferred to microtiter plate wells and absorbance level was measured at 490nm (OD_{490}) using a Precision Microplate Reader (Molecular Devices, USA). The assay was performed in triplicate for each MS genotype and was repeated one time.

3.5 Statistical Analysis

All comparisons, including descriptive statistics of the demographic data were analyzed using SPSS 17.0. Using the acid data obtained, Prism 7 software for Mac generated a pH curve for each genotype. The plateau pH, acid production rate, and half-time were calculated based on each curve. Percentages of xylitol-resistant MS genotypes in each treatment group at baseline and one year were compared by Chi-square test or Fisher's exact test. The plateau pH, acid production rate and half-time were compared between baseline and one year for the same as well as different treatment groups. For biofilm formation, means and standard deviations were calculated for each MS genotype. The normality of the data distribution was analyzed. For normal distribution, the Student's t-test was used, and non-parametric tests were used for abnormally distributed data. The biofilm formation was compared between baseline and one year for the same treatment group as well as between the two treatment groups.

4. Results

4.1 Subject Demographics and Bacteria Population

Of the 44 children enrolled in the study, 18 in the xylitol group and 11 in the placebo group completed the study. Among those who completed the study, eleven children in the xylitol group and seven children in the placebo group had MS infection. The mean±standard deviation of the age of these children was 17.8±9.0 months in the xylitol group and 15.9±10.8 months in the placebo group. In the xylitol group, 36% were females and 64% were males. In the placebo group, 57% were females and 43% were males. In both groups, the majority of the children were Hispanics (xylitol = 73%; placebo = 71%). Table 1 summarizes the characteristics of the 29 children that were included in this study. There were no significant differences in age, gender and ethnicity of the subjects between the two treatment groups.

Table 1 Study Population Demographics

	Xylitol Group	**Placebo Group**
Age	17.8±9.0 months	15.9±10.8 months
Sex	Male 64% Female 36%	Male 43% Female 57%
Race	Hispanic 73% Caucasian 9% African American 9% Mixed 9%	Hispanic 71% Asian 29%

With regards to dental caries experience, at baseline 2 of the 11 children in the xylitol group and none of the children in the placebo had caries. At one year, one of the 11 children in the xylitol group and 4 of the 7 children in the placebo group had caries.

In the xylitol group, at baseline eleven unique MS genotypes were isolated. At one year, sixteen unique MS genotypes were isolated. In the placebo group, at baseline six unique MS genotypes were isolated. At one year, nineteen unique MS genotypes were isolated. Table 2 presents a summary of the results.

Table 2 Dental Caries Experience

	Xylitol Group	**Placebo Group**
Baseline		
# Subjects	18	11
# Subjects with Caries	2	0
# dt/ total # of teeth	11/122	0/83
# ds/ total # tooth surfaces	24/518	0/356
MS levels (mean log CFU/ml ± SD)	2.20 ± 2.83	1.70 ± 1.82
Subjects with MS	4	4
# MS Genotypes	11	6
1 year		
# Subjects	18	11
# Subjects with Caries	1	2
# dt/ total # of teeth	1/202	5/125
# ds/ total # tooth surfaces	1/884	5/541
MS levels (mean log CFU/ml ± SD)	4.35 ± 1.93	4.38 ± 2.07
Subjects with MS	10	7
# MS Genotypes	16	19

4.2 Xylitol Sensitivity Assay

Xylitol sensitivity was determined by comparing the growth of MS genotypes in 1% glucose TPY broth with or without the presence of 1% xylitol. Examples of the bacterial growth curve of a xylitol-sensitive MS genotype and a xylitol-resistant MS

genotype are illustrated in Figure 1 and 2. For the xylitol resistant genotypes, the bacterial growth was not inhibited by xylitol in contrast to the xylitol-sensitive MS genotypes where growth inhibition was apparent (Figure 1 and 2).

Figure 1.

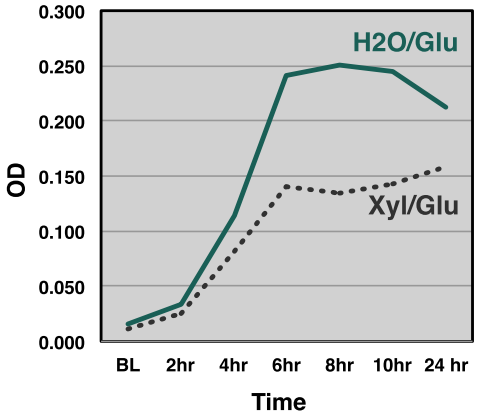

Figure 1. Bacterial growth curve of xylitol-sensitive MS genotype where growth is inhibited with the addition of xylitol.

Figure 2.

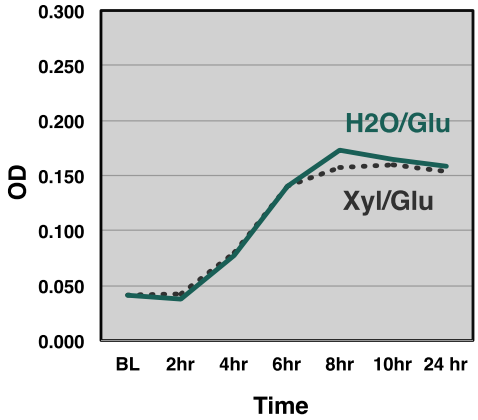

Figure 2. Bacterial growth curve of a xylitol-resistant MS genotype where growth is not inhibited with the addition of xylitol.

4.2.1. Genotype Level

At baseline, one of the eleven MS genotypes in the xylitol group was determined to be xylitol resistant while four of the six MS genotypes in the placebo group were defined as xylitol resistant (Figure 3). At one year, two of the sixteen MS genotypes in the xylitol group were considered to be xylitol resistant compared to three of the nineteen in the placebo group. There was no statistically significant difference in the presence of xylitol-resistant MS genotypes at baseline or one year between the two treatment groups

(Fisher's exact test, $p>0.05$).

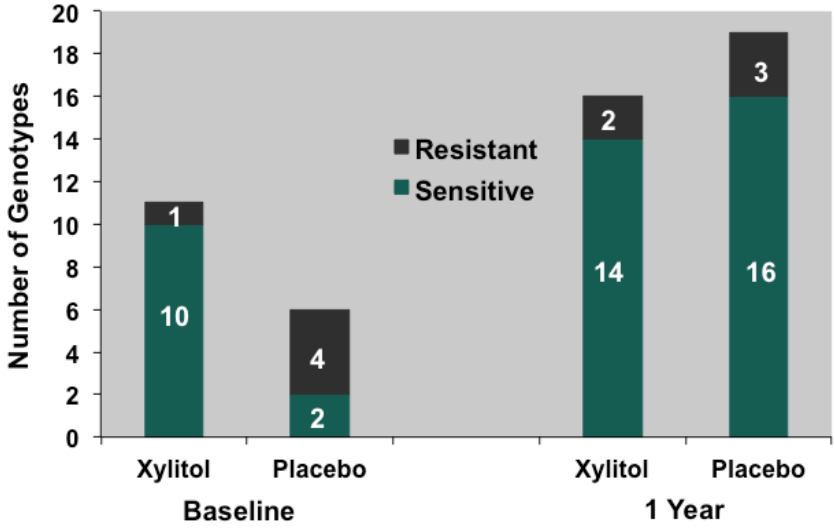

Figure 3. Distribution of Xylitol-Resistant vs. Xylitol-Sensitive MS Genotypes at Baseline and 1 Year. The number of genotypes in both xylitol and placebo groups were increased at one year compared to baseline, there were no significant changes in the relative number of xylitol resistant MS in the xylitol resistant MS in the xylitol group compared to the placebo group at 1 year ($p<0.05$).

4.2.2. Subject Level

Only descriptive statistics were calculated for the presence of xylitol-resistant MS at the subject level for both groups due to the small sample size at baseline. At baseline, 1 of the 4 subjects in the xylitol group and 2 of the 3 subjects in the placebo group had xylitol-resistant MS. In the xylitol group, the subject with the xylitol-resistant MS had dental caries. At one year, 2 of the 9 subjects in the xylitol group and 2 of the 7 subjects had xylitol-resistant MS (Figure 4). In both groups, 1 of the 2 subjects with the xylitol-resistant MS had dental caries. The distribution of xylitol-resistant MS was not

statistically significant between the two treatment groups at one year (Fisher's exact test, p=0.60).

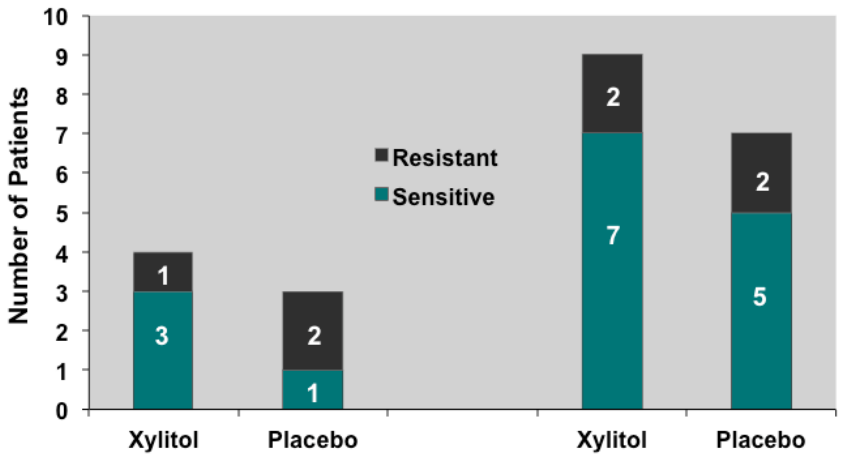

Figure 4. At subject level: Distribution of Xylitol-Resistant vs Xylitol-Sensitive MS Genotypes at Baseline and 1 Year (p>0.05).

4.3 Acid Production

For each MS genotype, a pH curve using a one-phase decay equation was plotted using Prism 7 software for Mac. Figure 5 shows an example of the pH curve of one MS genotype.

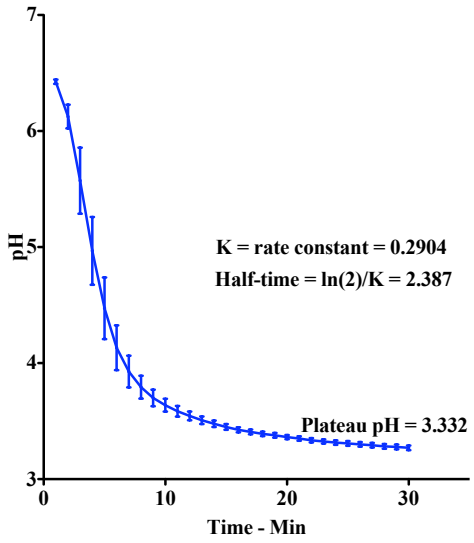

Figure 5. pH curve of one of the MS genotypes. The plateau pH, rate constant and half-time are calculated by Prism 7. The rate constant is the acid production rate. The half-time is ln(2)/K.

4.3.1. Xylitol vs Placebo Group

In the xylitol-group, the means ± standard deviations of the plateau pH were 3.69±0.11 at baseline and 3.44±0.20 at one year (ANOVA, $p<0.05$). In the placebo group, the means ± standard deviations of the plateau pH were 3.31±0.44 at baseline and 3.53±0.09 at one year (ANOVA, $p<0.05$). There was also a significant difference in plateau pH between the xylitol group at baseline and placebo group at 1 year (ANOVA, $p<0.05$). The results are summarized in table 4 and figure 6.

Table 4: Summary of Acid Production (Mean ± SE)

	Xylitol		Placebo	
	Baseline	**1 year**	**Baseline**	**1 year**
Plateau pH	3.69±0.03[a,c]	3.44±0.05[a]	3.31±0.18[b]	3.53±0.02[b,c]
Acid Production Rate (min^{-1})	0.22±0.01	0.15±0.02	0.19±0.04	0.18±0.01
Half-time (min)	3.39±0.25	5.77±3.90	5.90±2.16	4.22±0.34

The parameters with the same superscript are significantly different from each other ($p<0.05$).

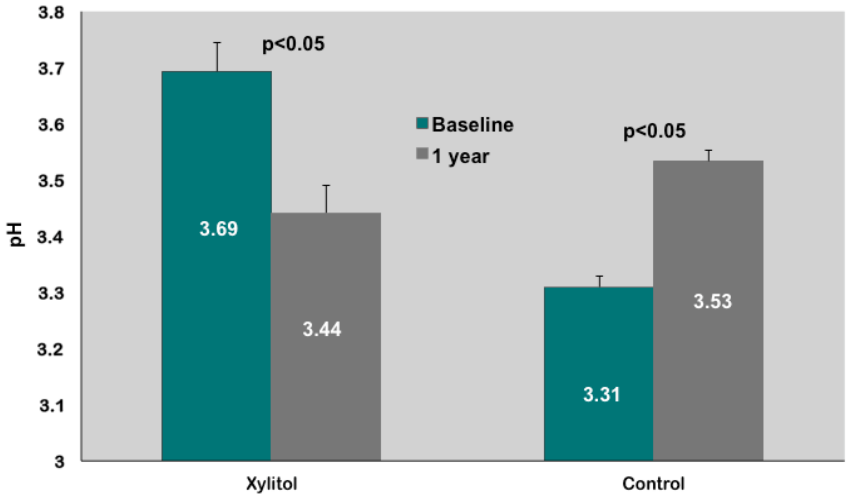

Figure 6. Plateau pH (Means ± SE) – Both within xylitol and placebo groups were significantly different at 1 year as compared to baseline ($p<0.05$).

In the xylitol-group, the means ± standard deviations of the acid production rate were 0.21±0.07 min^{-1} at baseline and 0.15±0.06 min^{-1} at one year. In the placebo group, the means ± standard deviations of the acid production rate were 0.19±0.11 min^{-1} at baseline and 0.18±0.06 min^{-1} at one year. For the half-time, in the xylitol-group, the means ± standard deviations were 3.39±0.83 min at baseline and 5.77±3.61 min at 1 year. In the placebo group, the means ± standard deviations for the half-time were 5.90±5.29 min at baseline and 4.22±1.47 min at 1 year. The acid production rate and half-time were not significantly different between nor within all groups (ANOVA, $p>0.05$).

4.3.2. Xylitol-Resistant vs Xyltiol-Sensitive Genotypes

When the resistant and sensitive strains were grouped together, the results were as follows. The means ± standard deviations of the plateau pH in the resistant strains and in the sensitive strains group were 3.31±0.37 and 3.56±0.14 respectively (ANOVA, $p<0.05$) as shown in figure 7.

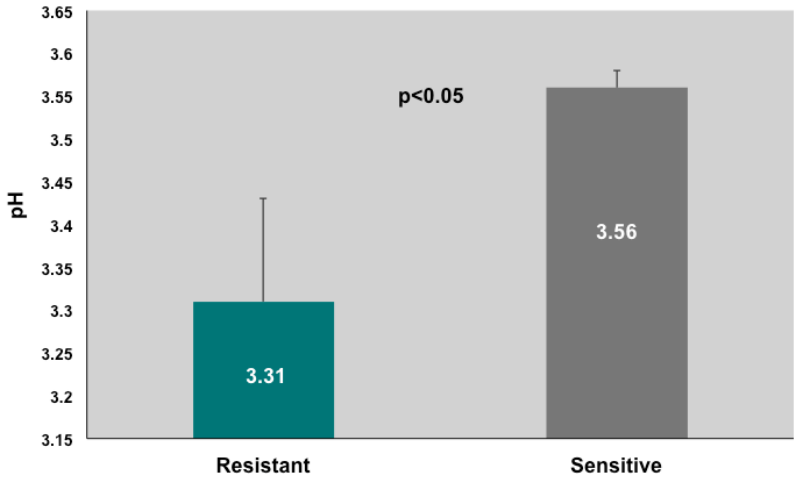

Figure 7. Plateau pH – Resistant vs. Sensitive Strains (Means ± SE). There is statistical difference between the resistant and sensitive strains group. ($p<0.05$)

The acid production rate in the resistant strains group was 0.19±0.10 min^{-1} and in the sensitive strains group was 0.18±0.06 min^{-1} (ANOVA, $p>0.05$). The half-time in the resistant strains group was 5.63±5.14 min and in the sensitive strains group was 4.71±2.11 min (ANOVA, $p>0.05$). Results are summarized in table 5.

Table 5: Summary of Acid Production Between Xylitol Resistant and Sensitive Strains (Mean ± SE)

	Resistant	**Sensitive**
Plateau pH	3.31±0.12*	3.56±0.02*
Acid Production Rate (min^{-1})	0.20±0.03	0.18±0.01
Half-time (min)	5.63±1.63	4.49±0.33

Parameter with the asterisk (*) is significantly different from each other ($p<0.05$)

4.3.3. Subject Level

When considering the dental caries experience, the means ± standard deviations of the plateau pH in the caries group and in the no caries group were 3.58±0.11 and 3.45±0.28 respectively. The acid production rate in the caries group was 0.20±0.05 min^{-1} and in the no caries group was 0.17±0.08 min^{-1}. The half-time in the caries group was

3.66±0.84 min and in the no caries group was 5.62±3.68 min. Table 6 summarized the data.

Table 6: Summary of Acid Production Between Caries and No Caries Group (Mean ± SE)

	Caries	**No Caries**
Plateau pH	3.58±0.02[a]	3.45±0.05[a]
Acid Production Rate (min^{-1})	0.20±0.01	0.17±0.01
Half-time (min)	3.66±0.17[b]	5.62±0.70[b]

The parameters with the same superscript are significantly different from each other ($p<0.05$).

Both the plateau pH and the half-time showed statistically significant differences between the caries versus no caries group (ANOVA, $p<0.05$) as shown in figure 8 and figure 9 respectively.

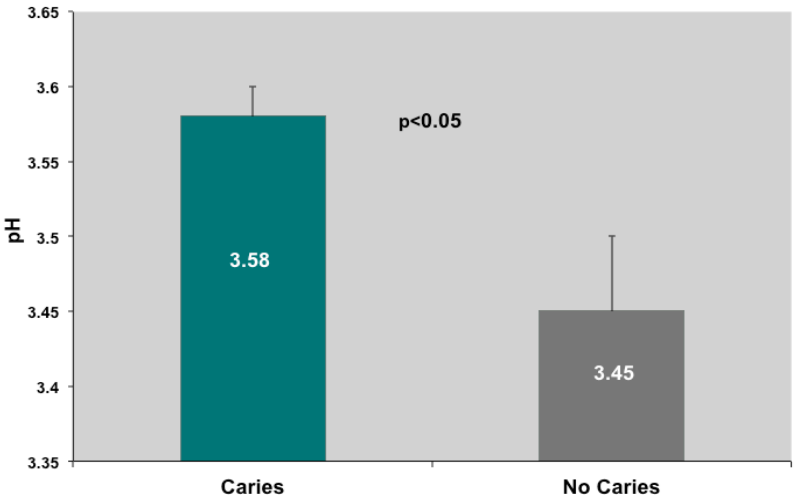

Figure 8. Plateau pH – Caries vs. No Caries (Means ± SE). There is statistical difference between the caries and no caries group ($p<0.05$).

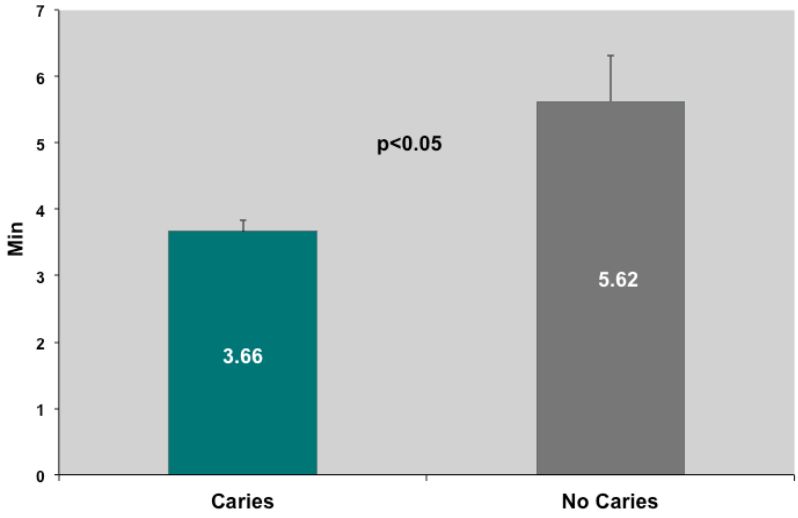

Figure 9. Half-Time – Caries vs. No Caries (Means ± SE). There is statistical difference between the caries and no caries group ($p<0.05$).

4.4 Sucrose-Dependent Biofilm Formation

4.4.1. Xylitol vs Placebo Group

In the xylitol-group, the means ± standard deviations of sucrose dependent biofilm formation were 0.09±0.03 OD_{490} at baseline and 0.08±0.01 OD_{490} at one year. In the placebo group, the means ± standard deviations of sucrose dependent biofilm formation were 0.09±0.03 OD_{490} at baseline and 0.08±0.02 OD_{490} at one year. There were no statistically significant differences in biofilm formation between the xylitol group and placebo group both at baseline and at 1 year as shown in figure 10.

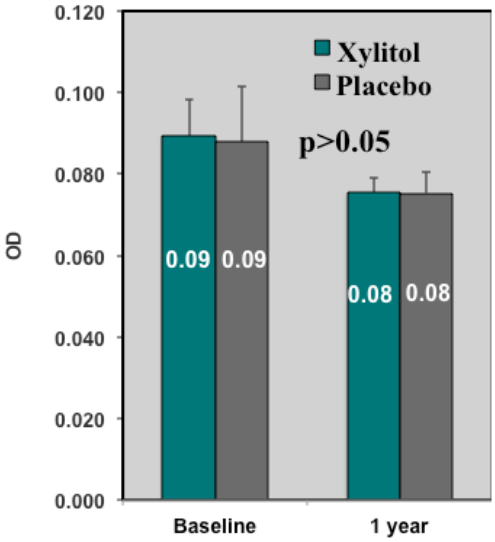

Figure 10. Biofilm formation: Xylitol vs. Placebo Group at Baseline and 1 year (Mean±SE). There are no statistical differences between the xylitol and placebo groups at baseline nor at 1 year ($p>0.05$).

4.4.2. Xylitol-resistant vs Xylitol-sensitive Group

When all the xylitol resistant genotypes were grouped together and compared to xylitol sensitive genotypes, there were also no statistically significant differences in biofilm formation (Student t-test, $p>0.05$) as shown in figure 11 (means ± standard deviations, Resistant =0.07±0.03 OD_{490}; Sensitive=0.08±0.02 OD_{490}).

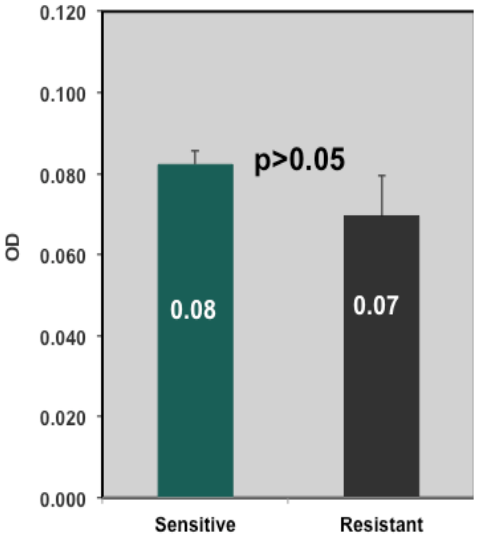

Figure 11. Biofilm formation: Sensitive vs. Resistant Group (Mean±SE). There is no statistical difference between the xylitol sensitive and resistant groups ($p>0.05$)

4.4.3. Subject Level

When considering the dental caries experience, the means ± standard deviations of sucrose dependent biofilm formation in the caries group and in the no caries group were 0.07±0.02 OD_{490} and 0.08±0.02 OD_{490} respectively. There was no statistically significant difference between the caries and the no caries group (ANOVA, $p>0.05$) as shown in figure 12.

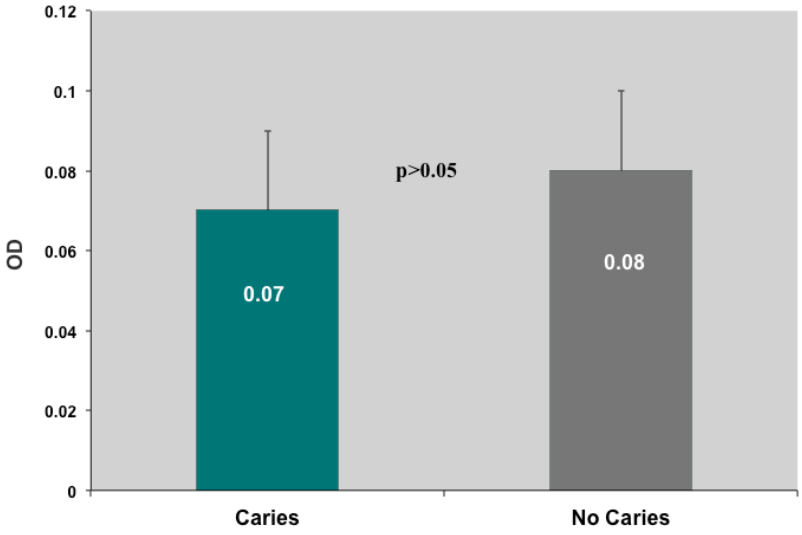

Figure 12. Biofilm Formation: Caries vs. No Caries Group (Mean±SE). There is no statistical different in biofilm formation between the caries and no caries group ($p>0.05$).

5. Discussion

5.1 Clinical Study

Our clinical study did not show a reduction in MS levels after one-year use of xylitol-wipe in children. This result is similar to some other long-term studies, defined as 12 months or more, even though most of the short-term studies with daily dose of greater than 6 grams of xylitol show a consistent reduction of MS[5-7, 32, 33]. Similar to our study,

Soderling et al. showed that after 24 months xylitol-gum use did not lead to the reduction of MS levels in the adult women[10]. Makinen et al.[9] studied the effect of xylitol gum and xylitol-sorbitol gum for 24 months on MS in 8-9 years old students with a 39-month follow-up and a no-gum control group. At 12 months, MS levels were decreased in all groups with no statistical differences between the groups. However, at 24 months, the levels of MS in plaque as well as saliva in both xylitol-gum group and xylitol-sorbitol-gum groups were significantly reduced as compared to the control group. Interestingly at 39 months, only the xylitol group had significantly reduced levels of MS compared to the other groups. In contrast, we found no change in MS levels in both groups at 12 months with no significant differences between the groups. The subjects in our study were much younger with a less mature oral flora than those in previous studies. In addition, our study was the first to use dental wipes as a delivery vehicle for xylitol. Therefore, it seems that subject age, duration and delivery vehicle of the xylitol impact the effect of xylitol on number of MS.

Despite the reports of inconsistent effects of xylitol use on levels of MS colonization[10, 34], and the lack of relationship between levels of MS relative to xylitol use, our study showed a significant reduction in the formation of new caries. This is similar to the study by Milgrom et al[15] where they showed that the use of xylitol syrup for one year in young children led to reduction of new dental caries. In our study, the lack of a significant difference between the numbers of MS in the xylitol group and the placebo group, though there was a significant reduction in caries in the xylitol group, suggests that xylitol may have selected MS with less virulence.

5.2 Xylitol Sensitivity

Two studies[18, 19] have suggested that long-term use of xylitol might select for xylitol-resistant MS, which may contribute to the caries preventive effect of xylitol. In the study by Trahan and Moutan[18], MS from adults and children who were xylitol consumers from 1.5 years to 10 years were compared to MS from adults and children who never consumed xylitol containing products. They found that 87% of the MS in the xylitol population were xylitol-resistant whereas only 10% in the control group were xylitol-resistant. A later study done by the same research group, Trahan et al[19] had similar conclusion that there is a positive correlation between xylitol consumption and the presence of xylitol-resistant MS strains. In contrast, we did not find an increase of xylitol-resistant MS strains after xylitol-wipe use compared to the placebo-wipe group.

The insignificant increase in number of xylitol-resistant MS strains after one year use of xylitol wipes in our study may be due to the following explanations. First, the degree of inhibition of MS by xylitol can be variable as demonstrated by Vadeboncoeur et al[35]. In their study, the growth of ten strains of MS was compared in the presence of 1% glucose to their growth with 1% glucose with 5% of xylitol. Their results showed that the degree of growth inhibition were different amongst ten strains ranging from 0-85%. Therefore, after xylitol exposure MS may have variations in xylitol-resistance even at a xylitol concentration much higher than the 1% of xylitol used in our study. It is therefore possible that our assay may not have picked up the MS strains with mild to moderate xylitol-resistance. Second, while the study by Trahan and Mouton that consisted of 6-59 years old subjects whose oral flora were relatively stable[18], our study

population were children aged 6-35 months whose oral flora were not fully established. This was shown by the more transient retention of MS genotype in our study population (data not presented in this thesis). These data suggested that xylitol-wipe use may increase the instability of the MS in young children instead of selecting xylitol-resistant MS. Lastly, an *in vitro* study has shown that different MS may require different exposure time to xylitol in order to become xylitol-resistant[16]. Therefore, in our study, some of the MS strains in the xylitol-wipe group may not have long enough exposure to xylitol to develop xylitol resistance.

5.3 Acid Production

Acid production by MS plays an essential part in dental caries development. It is well established that the acid production via fermentation of sugar by bacteria results in demineralization of hard dental tissues leading to loss of tooth structure.

In this study, we investigated the possibility that long-term xylitol use would alter the ability of MS to produce acid to explain an observed reduction in new carious lesions. Contrary to our hypothesis, the xylitol group had significantly lower plateau pH at 1 year as compared to baseline, which logically would lead to higher dental caries incidence. However, this lower final pH took longer to reach *in vitro* as the acid production rate in MS was observed to be reduced and the half-time increased. *In vivo*, the extended half-time may potentially allow the salivary buffering system to neutralize the acid before reaching the lower plateau pH.

The MS in the no caries placebo group also showed similar reduction in acid

production rate and longer half-time compared to the caries group with a lower plateau pH. The similarity between the MS in xylitol group and the no caries group suggest that one year use of xylitol wipes prevented dental caries through a decrease in acid production.

The reduction in acid production is similar to findings by Splieth et al.[23] and Twetman et al.[24] but different from the findings of a few previous studies[36-38]. Our data however, did not show that the xylitol resistant strains had less acid production abilities than the sensitive strains. This suggests that xylitol resistant strains may not be less virulent with regards to acid production abilities.

In summary, there is evidence in our study to support that use of xylitol for one year would result in selecting MS with decrease acid production ability and lead to less dental caries.

5.4 Biofilm

We also investigated whether using the xylitol wipes for one year would decrease sucrose-dependent biofilm formation, which may be closely related to MS colonization and cariogenicity. Our study showed no significant reduction in sucrose-dependent biofilm formation after one-year xylitol-wipe use. In addition, we did not find significant differences of sucrose-biofilm formation between xylitol-resistant strains and xylitol-sensitive strains. Literature on the effect of xylitol-use on sucrose-dependent biofilm formation of MS is very limited. Previously, Assev et al.[29] studied glucan formation of xylitol-sensitive and xylitol-resistant MS strains and found no significant difference

between them. This finding is consistent with our finding on xylitol-resistant and xylitol-sensitive MS in sucrose-dependent biofilm formation similar to a more recent study by Giertsen et al.[39]. However, a more recent study by Lee et al.[40] found that xylitol-resistant MS produced significantly less biofilm compared to the xylitol-sensitive MS. It is important to note that in their study, the xylitol-resistant MS were lab-induced unlike our study, where strains were obtained directly from subjects saliva. The lab-made xylitol-resistant MS may be different from naturally occuring strains possibly leading to the difference in results.

The colony morphology and gtfB gene expression of MS isolated from women after 12-months consumption of xylitol-gum were also compared to the control group in the study by Lee et al.[20]. They found that the MS from the xylitol group formed smaller, less undulated, and smoother colonies as well as decreased gtfB gene expression. However, there is no clear evidence of correlation of colony morphology with biofilm formation or glucan formation. In summary, there is no clear evidence to support that xylitol use leads to the inhibition of biofilm formation resulting in less new dental caries development.

6. Conclusion

Our xylitol-wipe study showed that one-year use of xylitol-wipe in young children significantly reduced new caries without altering MS colonization levels. The present study does not support the hypotheses that the use of xylitol-wipe for one year would select MS with 1) xylitol resistance and/or 2) reduced sucrose-dependent biofilm

formation in children. However, there is evidence to suggest that after one year xylitol use selected MS with decrease acid production ability in our patient population.

Effect of xylitol-wipe use may also play a role in other virulence factors of MS such as altered mutacin formation and sucrose-independent tooth colonization. In addition, xylitol may have an impact on other cariogenic bacteria such as lactobacillus, which further modify the caries risk in children. Therefore, additional studies to determine the effect of xylitol on other caries related microorganisms are needed. Understanding the mechanism of caries preventive effect of xylitol at the molecular level will help develop better recommendation and more targeted regimens for caries prevention in children.

7. Bibliography

1. Berkowitz RJ. Acquisition and transmission of mutans streptococci. J Calif Dent Assoc 2003;31(2):135-8.
2. Hamada S, Koga T, Ooshima T. Virulence factors of Streptococcus mutans and dental caries prevention. J Dent Res 1984;63(3):407-11.
3. Trahan L. Xylitol: a review of its action on mutans streptococci and dental plaque--its clinical significance. Int Dent J 1995;45(1 Suppl 1):77-92.
4. Ly KA, Milgrom P, Rothen M. Xylitol, sweeteners, and dental caries. Pediatr Dent 2006;28(2):154-63; discussion 92-8.
5. Holgerson PL, Sjostrom I, Stecksen-Blicks C, Twetman S. Dental plaque formation and salivary mutans streptococci in schoolchildren after use of xylitol-containing chewing gum. Int J Paediatr Dent 2007;17(2):79-85.
6. Loesche WJ, Grossman NS, Earnest R, Corpron R. The effect of chewing xylitol gum on the plaque and saliva levels of Streptococcus mutans. J Am Dent Assoc 1984;108(4):587-92.
7. Soderling E, Makinen KK, Chen CY, Pape HR, Jr., Loesche W, Makinen PL. Effect of sorbitol, xylitol, and xylitol/sorbitol chewing gums on dental plaque. Caries Res 1989;23(5):378-84.
8. Isotupa KP, Gunn S, Chen CY, Lopatin D, Makinen KK. Effect of polyol gums on dental plaque in orthodontic patients. Am J Orthod Dentofacial Orthop 1995;107(5):497-504.
9. Makinen KK, Alanen P, Isokangas P, Isotupa K, Soderling E, Makinen PL, et al. Thirty-nine-month xylitol chewing-gum programme in initially 8-year-old school children: a feasibility study focusing on mutans streptococci and lactobacilli. Int Dent J 2008;58(1):41-50.
10. Soderling E, Isokangas P, Pienihakkinen K, Tenovuo J. Influence of maternal xylitol consumption on acquisition of mutans streptococci by infants. J Dent Res 2000;79(3):882-7.
11. Stecksen-Blicks C, Holgerson PL, Olsson M, Bylund B, Sjostrom I, Skold-Larsson K, et al. Effect of xylitol on mutans streptococci and lactic acid formation in saliva and plaque from adolescents and young adults with fixed orthodontic appliances. Eur J Oral Sci 2004;112(3):244-8.
12. Makinen KK, Makinen PL, Pape HR, Jr., Peldyak J, Hujoel P, Isotupa KP, et al. Conclusion and review of the Michigan Xylitol Programme (1986-1995) for the prevention of dental caries. Int Dent J 1996;46(1):22-34.
13. Fontana M, Catt D, Eckert GJ, Ofner S, Toro M, Gregory RL, et al. Xylitol: effects on the acquisition of cariogenic species in infants. Pediatr Dent 2009;31(3):257-66.
14. Nakai Y, Shinga-Ishihara C, Kaji M, Moriya K, Murakami-Yamanaka K, Takimura M. Xylitol gum and maternal transmission of mutans streptococci. J Dent Res;89(1):56-60.
15. Milgrom P, Ly KA, Tut OK, Mancl L, Roberts MC, Briand K, et al. Xylitol pediatric topical oral syrup to prevent dental caries: a double-blind randomized clinical trial of efficacy. Arch Pediatr Adolesc Med 2009;163(7):601-7.

16. Trahan L, Bourgeau G, Breton R. Emergence of multiple xylitol-resistant (fructose PTS-) mutants from human isolates of mutans streptococci during growth on dietary sugars in the presence of xylitol. J Dent Res 1996;75(11):1892-900.
17. Waler SM, Rolla G, Assev S, Ciardi JE. The effect of xylitol on plaque metabolism. Swed Dent J 1984;8(3):155-61.
18. Trahan L, Mouton C. Selection for Streptococcus mutans with an altered xylitol transport capacity in chronic xylitol consumers. J Dent Res 1987;66(5):982-8.
19. Trahan L, Soderling E, Drean MF, Chevrier MC, Isokangas P. Effect of xylitol consumption on the plaque-saliva distribution of mutans streptococci and the occurrence and long-term survival of xylitol-resistant strains. J Dent Res 1992;71(11):1785-91.
20. Lee YE, Choi YH, Jeong SH, Kim HS, Lee SH, Song KB. Morphological changes in Streptococcus mutans after chewing gum containing xylitol for twelve months. Curr Microbiol 2009;58(4):332-7.
21. Kakuta H, Iwami Y, Mayanagi H, Takahashi N. Xylitol inhibition of acid production and growth of mutans Streptococci in the presence of various dietary sugars under strictly anaerobic conditions. Caries Res 2003;37(6):404-9.
22. Campus G, Cagetti MG, Sacco G, Solinas G, Mastroberardino S, Lingstrom P. Six months of daily high-dose xylitol in high-risk schoolchildren: a randomized clinical trial on plaque pH and salivary mutans streptococci. Caries Res 2009;43(6):455-61.
23. Splieth CH, Alkilzy M, Schmitt J, Berndt C, Welk A. Effect of xylitol and sorbitol on plaque acidogenesis. Quintessence Int 2009;40(4):279-85.
24. Twetman S, Stecksen-Blicks C. Effect of xylitol-containing chewing gums on lactic acid production in dental plaque from caries active pre-school children. Oral Health Prev Dent 2003;1(3):195-9.
25. Lif Holgerson P, Stecksen-Blicks C, Sjostrom I, Twetman S. Effect of xylitol-containing chewing gums on interdental plaque-pH in habitual xylitol consumers. Acta Odontol Scand 2005;63(4):233-8.
26. Napimoga MH, Hofling JF, Klein MI, Kamiya RU, Goncalves RB. Tansmission, diversity and virulence factors of Sreptococcus mutans genotypes. J Oral Sci 2005;47(2):59-64.
27. Kuramitsu HK. Virulence factors of mutans streptococci: role of molecular genetics. Crit Rev Oral Biol Med 1993;4(2):159-76.
28. Wunder D, Bowen WH. Action of agents on glucosyltransferases from Streptococcus mutans in solution and adsorbed to experimental pellicle. Arch Oral Biol 1999;44(3):203-14.
29. Assev S, Stig S, Scheie AA. Cariogenic traits in xylitol-resistant and xylitol-sensitive mutans streptococci. Oral Microbiol Immunol 2002;17(2):95-9.
30. Zhan L, Cheng, J., Chang, P., Ngo, M., DenBesten, P., Hoover, C. Effect of Xylitol Wipes on Cariogenic Bacteria and Caries in Young Children. J. Dent. Res. 2012.
31. Yoshida A, Kuramitsu HK. Multiple Streptococcus mutans Genes Are Involved in Biofilm Formation. Appl Environ Microbiol 2002;68(12):6283-91.

32. Ly KA, Riedy CA, Milgrom P, Rothen M, Roberts MC, Zhou L. Xylitol gummy bear snacks: a school-based randomized clinical trial. BMC Oral Health 2008;8:20.
33. Wennerholm K, Arends J, Birkhed D, Ruben J, Emilson CG, Dijkman AG. Effect of xylitol and sorbitol in chewing-gums on mutans streptococci, plaque pH and mineral loss of enamel. Caries Res 1994;28(1):48-54.
34. Soderling E, Isokangas P, Pienihakkinen K, Tenovuo J, Alanen P. Influence of maternal xylitol consumption on mother-child transmission of mutans streptococci: 6-year follow-up. Caries Res 2001;35(3):173-7.
35. Vadeboncoeur C, Trahan L, Mouton C, Mayrand D. Effect of xylitol on the growth and glycolysis of acidogenic oral bacteria. J Dent Res 1983;62(8):882-4.
36. Miyasawa-Hori H, Aizawa S, Takahashi N. Difference in the xylitol sensitivity of acid production among Streptococcus mutans strains and the biochemical mechanism. Oral Microbiol Immunol 2006;21(4):201-5.
37. Scheie AA, Fejerskov O, Danielsen B. The effects of xylitol-containing chewing gums on dental plaque and acidogenic potential. J Dent Res 1998;77(7):1547-52.
38. Giertsen E, Emberland H, Scheie AA. Effects of mouth rinses with xylitol and fluoride on dental plaque and saliva. Caries Res 1999;33(1):23-31.
39. Giertsen E, Arthur RA, Guggenheim B. Effects of xylitol on survival of mutans streptococci in mixed-six-species in vitro biofilms modelling supragingival plaque. Caries Res;45(1):31-9.
40. Lee SH, Choi BK, Kim YJ. The cariogenic characters of xylitol-resistant and xylitol-sensitive Streptococcus mutans in biofilm formation with salivary bacteria. Arch Oral Biol.

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