Title
Effect of Povidone-iodine foam in children with active decay

Author
Kim, Ji Young

Publication Date
2008

Peer reviewed|Thesis/dissertation

Effect of Povidone-iodine Foam in Children with Active Decay

by

Ji Young Kim, DDS

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

ACKNOWLEDGEMENTS

I would like to thank my research and thesis mentor, Dr. John Featherstone for your time, guidance and encouragement in this project. I also would like to thank Dr. Pamela DenBesten, Dr. Chuck Hoover, and Dr. Stuart Gansky for being part of my committee; Dr. Ling Zhan for her guidance and support throughout this project. I'd also like to acknowledge that this thesis is a continuation of a clinical research project begun by Dr. Purvi Zavery as part of her residency training in UCSF Pediatric Dentistry and portions of the Literature Review are attributable to her. This research was supposed by a UCOP Pacific Rim grant as well as an IADR/GSK innovative research award. I would also like to thank Omnii Oral Pharmaceuticals for supplying the product at no charge.

ABSTRACT

Effect of Povidone-iodine Foam in Children with Active Decay.

Kim J*, Featherstone JDB, DenBesten PK, Hoover CI, Zhan L, Gansky SA

(University of California, San Francisco, CA)

Purpose: To determine the efficacy of four weekly applications of a povidone iodine/ fluoride (PVPI-F) foam on the reduction of mutans streptococci (MS), *Lactobacillus* (LB), and caries in children with active caries using a controlled randomized double blinded clinic trial.

Methods: Sixty 6-9 year olds with 1-6 frank dental caries were randomized to the F-only or PVPI-F group blinded as group A and B. Four weekly foam applications were performed at baseline and 6 month visit. Saliva samples were taken at baseline (S1), 2 months (S2), 6 months (S3) and 1 year follow-up visit (S4). MS, LB and total viable bacterial count (TVC) were enumerated on selective media. dmfs/DMFS data were collected using modified WHO criteria at the initial and final visits.

Results: There was no statistically significant difference for new caries at 1 year between the two groups although there was a trend toward a decrease in new interproximal ss-ds/DS and new total ss-ds/DS in the PVPI-F group. There was no significant change of bacterial levels between baseline and 1 year in each group. It was also interesting to note that an increase in $\log_{10}$LB was positively correlated with increased interproximal ds/DS (Pearson's correlation, r=0.40). Subjects with reduced $\log_{10}$MS showed significantly less new total caries surfaces and interproximal caries surfaces. Subjects with reduced $\log_{10}$TVC showed more new pits and fissure ds/DS and close-to-statistically-significant less number of total new caries surfaces.

Conclusions: In this randomized double-blinded study, PVPI-F foam was ineffective in significantly reducing cariogenic bacterial counts or dmfs/DMFS in 6- to 9-year-old children with active caries.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

Effect of Povidone-iodine Foam in Children with Active Decay

1. Literature Review

1.1. Epidemiology of Dental Caries

Dental caries is a pathological and multi-factorial process that involves the dynamic integration of oral bacteria, saliva, enamel, dietary substrates, and many other factors. While dental caries is still very common, there is a skewed distribution of caries prevalence. Despite many efforts, there has been a plateau in the reduction of caries since the 1990s.

Today, there are still a significant number of children who suffer from dental caries and even rampant early childhood caries[1]. In fact, dental caries is the most common chronic disease of childhood, 5 times more common than asthma[2].

There is a skewed distribution of caries prevalence in many western countries, and the Unites States is no exception to this rule. A minority of people, who are often in low socioeconomic communities, accounts for the majority of caries experience in the United States and remains at high risk of developing caries. According to a national survey conducted in the 1980s, by age 17 years, 40 percent of the population accounted for 80 percent of the caries

in school-age children[3]. Additionally, data from the Third National Health and Nutrition Examination Survey (NHANES), a survey conducted between 1988 and 1994, found that for 2-5 years children, 75 percent of dental caries (primary dention) was found in 8.1 percent of the population[4,5]. Kaste *et al.* affirmed this fact by showing that approximately 25% of children and adolescents in the 5- to 17-year age range accounted for about 80% of the caries experienced in the permanent dentition[6].

There has been an overall decrease in caries prevalence experienced over the last 30-40 years. It is believed that the earlier caries reduction of 40 to 70 percent is most notably due to the incorporation of fluoride in toothpastes and public water supplies since the mid-1970s[7,8]. However, since the1990s, we have witnessed a plateau in the reduction of caries[9]. In fact, according to the NHANES 1999-2004 survey, dental caries in primary teeth has increased for children aged 2-5 years since the NHANES 1988-1994 survey[5].

What is also distressing is that untreated dental decay continues to persist regardless of the cutting-edge restorative techniques and dental materials in the United States. According to a Dental Health Foundation report that presented data collected in 1993 and 1994, 53% of 6- to 8-year-olds in California have

untreated tooth decay[10]. Such untreated tooth decay may lead to considerable suffering that is often alleviated only by the loss or extraction of the infected tooth[11]. Oral health status is closely associated with quality of life in that dental caries may result in impairment (the loss of teeth), functional limitation (difficulties in chewing and speaking), and discomfort (a toothache) as well as undermine self-image, self-esteem and social acceptance[12,13].

1.2. Etiology of Dental Caries

1.2.1. Microbiology

Dental caries is the most common infectious and transmissible disease inflicting humans today. Among the numerous oral bacteria, mutans streptococci (MS) and *Lactobacillus* (LB) species are known to be the two major bacterial groups that cause dental caries in humans[14,15]. The category MS refers to a collection of bacterial species including *Streptococcus mutans* and *Streptococcus sobrinus*. MS are believed to colonize the mouth even before eruption of teeth and initiate the dental caries process within the enamel[16,17].

Unlike MS which is known to be the initiator of a carious lesion in the enamel, various LB species are believed to be important secondary cariogenic pathogens involved in the progression of decay further into the tooth structure.

LB, being highly acidogenic, produces lactic acid from fermenting glucose and other carbohydrates. LB are difficult to eradicate since they can colonize various oral surfaces including the teeth, tongue and cheeks. High counts of LB in saliva and plaque are often indicative of high total carbohydrate consumption and are , therefore, considered an indirect indicator of caries risk[18].

Studies have demonstrated that high salivary MS and LB levels were associated with increased risk of developing caries[19,20,21] and that counts of MS and LB correlated positively with DMFT scores[22,23]. Ramos-Gomez *et al.* concluded that children with $\log_{10}$ MS $\geq$ 3.0 and/or $\log_{10}$ LB $\geq$1.5 were about 5 times as likely to have early childhood caries (ECC) than those with lower cariogenic bacterial levels[24]. In Kohler and Bratthall's study, they found that caries-free children had less than 10,000 colony forming unit/ml (CFU/ml) of cariogenic bacteria in saliva[25].

In addition, Keene *et al.* and Mundorff *et al.* found that there is a positive correlation between MS levels in saliva with those in pooled plaque from various sites in the mouth[26,27]. MS levels can be a predictor for an individual's caries risk[28]. Even though it is not feasible to predict caries risk precisely solely based

on bacterial counts, bacterial counts are important tools in estimating caries risk—especially in predicting low caries risk[29].

1.2.2. Transmission of Cariogenic Bacteria

Children most often acquire MS from their caregivers as the bacteria are transmitted via saliva, and this is known as vertical transmission[1]. Mothers who have untreated dental decay seem to be at greater risk to transmit the pathogenic microorganisms than those without dental decay[30]. In a study conducted by Kohler *et al.*, they were able to inhibit or delay the establishment of MS in infants by reducing the salivary level of MS in their highly infected mothers through treating active carious lesions and providing topical chlorhexidine gels[31].

More recent reports indicated microbes can be transmitted between members of a group, and this is known as horizontal transmission[32,33]. Van Loveren *et al.* found that even when a child acquires MS after the age of 5, there may be similarity between MS in mother, father, and child[32]. This indicates that horizontal transmission can occur among family members. Mattos-Graner *et al.* found the presence of matching genotypes of *S. mutans* among children attending one nursery school[33].

1.2.3. Demineralization and Remineralization

The dental enamel, which consists of carbonated apatite, is dissolved by organic acids (lactic and acetic) that are produced by the cellular action of plaque bacteria in the presence of fermentable carbohydrates. At a low pH, when the oral environment is undersaturated with mineral ions, relative to a tooth's mineral content, these acids travel into the tooth and dissolve calcium and phosphate from the tooth mineral. This process is known as demineralization. If demineralization is allowed to continue, it eventually produces a cavitation in the tooth structure. The partially demineralized tooth can be repaired by a process called remineralization in which the subsequent loss of calcium, phosphate, and fluoride ions to be replaced by fluorapatite crystals. These crystals are more resistant to enamel breakdown by the resident organic acids and are substantially larger than the original crystals. The initial demineralization process can be reversed or inhibited by saliva, which contains minerals, fluoride, protective proteins and antibacterial reagents. Demineralization and remineralization occur numerous times everyday[1].

1.2.4. Host Factors and Modifying Factors

The delicate balance between caries progression and its reversal is determined by many factors including the quantity and quality (virulence) of the

bacteria present in the mouth, the contents and flow rate of saliva, the diet as well as the use of xylitol, fluoride, and antimicrobial agents such as chlorhexidine[1]. These factors can have a direct effect on the balance of protective and pathogenic factors, possibly increasing or decreasing a patient's caries risk.

1.2.4.1. Saliva

The important role that saliva plays on oral health is well-documented. It is one of the key host defense systems in caries development and progression. Saliva contains antibacterial, antiviral, and antifungal proteins as well as proteins, phosphates, and bicarbonate molecules that have a buffering capacity. It also clears foods and bacteria. All of these help maintain the oral environment at an optimum pH of 7, thereby effectively neutralizing acids and decreasing the mineral dissociation of teeth[34]. The breakdown of fermentable carbohydrates by cariogenic bacteria, namely MS and LB, causes a decrease in the salivary pH and creates a gradient allowing acid to enter the tooth. Then, Ca and PO_4 ions leach out of the enamel rods of the tooth, forming a demineralized zone[35]. If the pH of the saliva is not kept sufficiently high, the teeth may erode or be susceptible to caries formation.

1.2.4.2. Diet

Oral cariogenic bacteria convert sugars (glucose and fructose, and most commonly sucrose) into acids such as lactic acid through a glycolytic process called fermentation[35]. Therefore, the quantity and frequency of carbohydrate consumption can affect the caries challenge. Even in the presence of cariogenic bacteria, if little or no fermentable carbohydrates are present, little or no acid will be produced. In such cases, the risk of caries development will be reduced.

Frequency of consumption of fermentable carbohydrates can also influence caries challenge. Increased frequency of carbohydrate ingestion increases the acidity of plaque and causes a prolonged acidic environment in the mouth. This increases the potential for enamel demineralization and, in turn, provides inadequate time for remineralization by various components of saliva. In Arcella and coworkers' study, a statistically significant relationship was found between DMFT and eating frequency. The frequency of "high sugars and high starch events" accounted for 8% of the DMFT variance, and overall frequency of separate eating events accounted for 18% of the DMFT variance[36].

1.3. Management of Dental Caries

Clinical management of dental decay usually consists of physical removal of decay from the tooth and restoration of the defect. This type of surgical

approach has been the predominant mode of caries management for the past 150 years. However, removal of decay and placement of restorations only have a short-term effect of decreasing levels of MS and LB: cariogenic organisms rapidly re-establish throughout the mouth and gradually return their levels to the pre-treatment levels[37]. Cariogenic bacteria or caries risk cannot be reduced or eliminated solely by dental restorative work.

It is logical to think that antibacterial agents should be used in managing caries since dental caries is bacterial in etiology. Even though there are no antibiotics or vaccines against dental caries, the following antimicrobial agents have been used and studied in efforts to control intraoral bacterial levels.

1.3.1. Fluoride, Xylitol, and Chlorhexidine

Fluoride has been utilized in dentistry since the 1940s and is known for its well-established effects on the remineralization process[1]. However, it can also act as an inhibitor of cariogenic bacteria. Fluoride cannot enter the bacterial cell as an ion, but can readily diffuse into the cell as HF. When the bacteria produce acid, the hydrogen ion from the acid combines with the fluoride ion present in the plaque to form HF, which diffuses into the bacterial cell. Inside the cell, the HF dissociates and releases a fluoride ion, which interferes with enzymatic pathways

of the bacteria. Fluoride interferes with enolase, a critical enzyme in the bacteria's metabolic pathway and reduces the acid by-product[38]. However, fluoride's effectiveness as a antimicrobial agent has been shown to be very limited, and a high bacterial challenge cannot be completely overcome by even high-concentration fluoride therapy[39].

Xylitol is a five-carbon sugar alcohol that is used as a sugar substitute. Xylitol is a naturally occurring sweetener found in the fibers of many fruits and vegetables, including various berries, plums, pears, corn husks, oats, and mushrooms[40]. Xylitol is roughly as sweet as sucrose but with only two-thirds the food energy.

In vitro, growing MS on dietary sugars in the presence of xylitol or on xylitol only results in the selective enrichment of xylitol-resistant mutants that lack a constitutive fructose-PTS activity and are unable to accumulate toxic xylitol phosphate. These xylitol-resistant mutants are associated with caries prevention[41].

Xylitol also disturbs the protein synthesis of MS, thereby decreasing the bacteria's ability to adhere to a tooth surface[42]. Xylitol can also combine with calcium in aqueous solution. Thus, xylitol might act as a Ca^{2+} ion carrier

supplying Ca^{2+} to enamel for remineralization[43]. Moreover, xylitol in the form of chewing gum increases natural salivary buffering capabilities against caries by increasing salivary flow and aids in caries prevention[44].

Chlorhexidine is a positively charged bis-biguanide antiseptic that is bactericidal to both gram-positive and gram-negative microbes[45]. It is also bacteriostatic. Its positive charge allows it to bind to the enamel pellicle, hydroxyapatite and bacterial cell walls. When bound to the bacterial cell wall, it disrupts cell membranes by binding to their negatively charged sites[46].

Currently in the United States, 0.12% chlorhexidine gluconate oral rinse containing ethyl alcohol is available by prescription. It has been shown to reduce MS to low levels in saliva and dental plaque in children and adults with high caries risk. However, its efficacy on *LB* has been shown to be very limited[47]. Some disadvantages of chlorhexidine include its unpleasant taste, temporary tooth staining, formation of supra-gingival calculus, and temporary loss of sense of taste.

1.3.2. Povidone-iodine

Povidone-iodine (PVPI) is a water-soluble complex of iodine with polyvinylpyrrolidone (PVP) and is known to be a powerful broad-spectrum

antibacterial agent, effective against a wide range of bacteria, viruses, fungi, protozoa and spores[48]. It is widely used in hospitals for cleansing and disinfecting the skin, preparing the skin preoperatively and treating infections susceptible to iodine. Allergic reactions to PVPI occur in the general population at a rate of 1 person in 1000. The adverse effects include irritation, sensitivity, skin burns, bad taste and staining[49]. However, there is sufficient evidence showing that PVPI is safe for use as a topical antimicrobial agent[50].

Povidone-iodine (PVPI) is an iodophor consisting of a complex of iodine (I) with polyvinyl pyrrolidone (PVP) surfactant[50]. PVP is combined with I so that its ability to dissolve in water increases, its irritability is reduced, and the staining caused by pure iodine is minimized. When a small amount of free iodine is liberated from the PVP complex in the presence of water, it becomes biologically active and asserts its germicidal action. It works through disruption of pathogen cell walls[51].

In dentistry, topical application of iodine solutions has demonstrated suppression of oral *S. mutans* populations. In the study of Gibbons *et al.*, efforts to disinfect tooth surfaces by application of 0.2% potassium iodine (KI) solution showed that the populations of *S. mutans* on some tooth surfaces could be

affected for up to 13 weeks after treatment[52]. In 1979, Caufield *et al.* demonstrated that a prophylaxis followed by 3 topical applications of a 2% iodine-potassium iodide solution (I_2-KI) could significantly reduce the levels of *S. mutans* in fissure and proximal plaques and in saliva for 20 to 24 weeks[53].

More recently, a randomized placebo-controlled clinical study by Lopez *et al.* assessed the efficacy of 10% PVPI solution therapy in the prevention of early childhood caries. Eighty-three caries-free subjects aged 12 to 19 months with unremarkable medical history but with high caries risk were given PVPI solution applications every 2 months for a year. It was found that topical antimicrobial therapy using PVPI increased disease-free survival and reduced the incidence of dental caries in pediatric dental patients with a high caries risk[54].

In the pilot study conducted by Amin *et al.*, a 10% PVPI solution was applied 3 times at 2-month intervals to the teeth of high caries risk children who underwent dental rehabilitation under general anesthesia (GA). All children's *S. mutans* levels decreased significantly at 6 months regardless of the use of PVPI. It was concluded that extensive one-time restorative dental treatment, not PVPI applications, resulted in a significant reduction of *S. mutans* levels at 6 months following dental rehabilitation under GA. At 1 year, 5 of 8 children in the control

group, who received no PVPI applications, developed new caries compared to only 2 of 11 children in the experimental group, who received 3 PVPI applications[51].

A similar study completed by Zhan *et al.* with 20 children showed that a single application of PVPI solution following dental rehabilitation under GA in children under 5 years of age reduced MS and LB levels significantly for up to 3 months[55]. However, in contrast to the Amin *et al.*'s study, the 1-time application did not reduce caries incidence at 1 year. This supports the notion that multiple applications of 10% PVPI could possibly decrease future caries incidence by reducing the two major cariogenic bacterial groups, namely MS and LB, in the oral cavity of children with ECC.

Another pilot study conducted at UCSF using PVPI foam on high caries risk children suggested trends of MS and LB levels reduction at 1 week compared to baseline, but these effects were diminished by 1 month (unpublished data). The PVPI foam formulation was accepted well by the participating children. These results suggest that 4 PVPI foam treatments repeated at weekly intervals may be a good treatment regimen to substantially reduce or eliminate MS and LB.

2. Significance of the Study

The prevalence of dental decay shows that we need an antibacterial agent to overcome the bacterial challenge in a high caries risk patient. PVPI has the potential to be efficacious against these cariogenic bacteria. Furthermore, the beneficial effects of a high concentration F (5000 ppm F) foam or gel are well-known in enhancing remineralization of carious lesions. Chemically, PVPI and F can be combined to enhance the antibacterial effect of PVPI and the remineralizing effect of fluoride. Potentially a PVPI-F combined foam could be applied to the teeth of children at appropriate intervals throughout a year, reducing both the MS and LB bacterial challenge, enhancing remineralization, and markedly reducing or even eliminating new caries formation. The advantages expected by using this antibacterial and fluoride combination in the form of a flavored foam are improved taste and easier application for routine treatment, which may broaden its future use in dental practice or home dental care for caries prevention in children with high caries risk. PVPI foam is expected to have the same efficacy as the previously used PVPI solution.

3. Specific Aim

The aim of the study was to conduct a year-long randomized clinical trial to determine the efficacy of 4 weekly applications of a novel PVPI-F foam at baseline and 6 months on the reduction of oral cariogenic bacteria levels, namely MS and LB species as well as caries increments in children with active caries.

4. Hypothesis

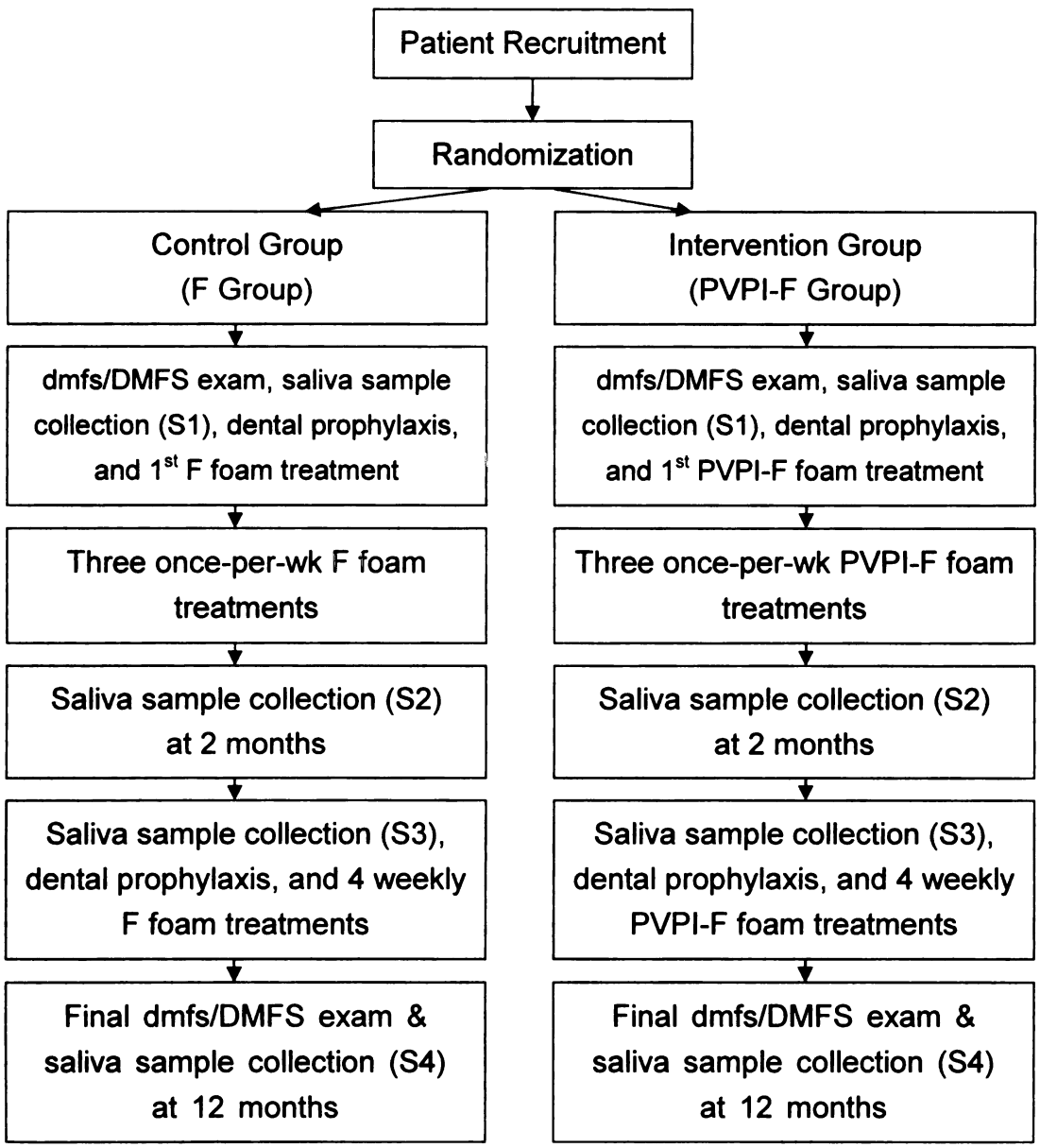
We hypothesized that twice yearly 4 weekly applications of PVPI-F would suppress MS and LB colonization, decrease the genetic diversity of MS, enhance remineralization and reduce future caries formation.

If the hypothesis is proven to be true, it will indicate that PVPI-F foam may be a convenient antibacterial treatment as a strategy in caries prevention for children, especially for those with high caries risk coming from low socioeconomic families.

5. Experimental Design and Methods

5.1. Overview of Study- Flow Diagram

dmfs	decayed, missing and filled surfaces in primary dentition
DMFS	decayed, missing and filled surfaces in permanent dentition
PVPI-F	povidone iodine/fluoride
F	Fluoride



5.2. Subject Recruitment, Randomization and Blinding

The UCSF Committee on Human Subject Research (CHR) approval was obtained prior to recruitment of any participants (CHR#: H9136-26344-01A; approved on 01/18/05). Parents or legal guardians of qualified subjects received a verbal and written explanation of the study procedures and protocol (see appendix for forms). Consenting parents read and signed the informed consent form previously approved by the UCSF CHR. Any questions regarding the study including the nature and details of the study were thoroughly answered by the study recruiters. Children who agreed to participate in the study read and signed the informed assent document previously approved by the UCSF CHR (see appendix for forms).

Other than the collection of saliva samples, all study procedures were identical to those that the participants would otherwise have received at a typical dental office. There was no extra cost to the subjects for participating in this study.

The study population was recruited from the patients in the Postdoctoral as well as the Pre-doctoral Pediatric Dental Clinics in the Division of Pediatric Dentistry at UCSF.

A total of 60 healthy 6- to 9-year-olds with 1 to 5 frank carious lesions consented to be subjects in the study.

The inclusion criteria included patients who were:

a) registered at the UCSF Pediatric Graduated Dental or Predoctoral Dental Clinic;

b) 6 to 9 years old; with a dental history of at least 1 active dental cavity within the last year;

c) residing in or within a 25 mile radius of San Francisco.

The exclusion criteria included children who had:

a) serious chronic systemic diseases, such as AIDS, diabetes, etc., or a medical history which includes antibiotics or medicines taken within the past 3 months that will affect their oral flora;

c) periodontal diseases that require regular periodontal treatment; and

d) dry mouth or difficulty spitting.

A randomization schedule created by a computer program was used to assign 60 participants to either a control: F foam group or intervention: PVPI-F foam group. Details of the products used are described below. The investigators rendering foam treatment assigned each subject to either group

based on the randomization table. Both F (5000 ppm F) and PVPI-F foam (1% active iodine [I] and 5000 ppm F) were provided by Omnii Oral Pharmaceuticals (West Palm Beach, FL, U.S.) with similar appearance and labeled as Group A and B. Only the study PI and statistician knew the group assignment.

Fifteen milliliter sterile conical tubes were used to collect saliva samples, and they were uniquely marked with the numeric codes and 2- or 3-letter initials assigned to individual subjects, the date of collection, and the saliva sample number (1, 2, 3 or 4 corresponding to the 4 collections for each subject twice during their year in the study). The corresponding foam treatment was performed at baseline and then once a week for 3 consecutive weeks. The 4 weekly foam treatments were repeated 6 months after baseline. Stimulated saliva samples were collected at baseline before the foam treatment, at 1 month, 5 months, and 1 year after the first 4 weekly treatments. The investigators coordinated with the Pre-doctoral and the Postgraduate Pediatric Dental Clinics to have all carious lesions of the enrolled subjects treated within 6 months after enrollment as they are indicated in the conventional treatment plan.

5.3. Clinical Examination

All dental exams on the subjects were performed at baseline and 1 year after enrollment at the UCSF Postgraduate Dental Clinic with the standard dental setting. First, the patient's medical history was thoroughly reviewed. Then, either the presence of at least 1 carious lesion or the subject's history of having at least 1 carious lesion within the past 1 year was confirmed. A thorough dental exam was carried out by 1 examiner, who used an intraoral mirror and a dental explorer as well as bilateral bitewing and periapical radiographic films.

The following information was recorded on a standard dental charting form for each subject: teeth present, carious surfaces, restored surfaces and teeth missing as a result of dental decay. Standard dmft/DMFT and dmfs/DMFS indices were calculated using NIDCR-modified WHO criteria (WHO, 1997) at baseline and at 1 year by the same examiner with the aid of radiographs. Lowercase letters were used to specify the primary dentition (dmft: decayed, missing, and filled teeth; dmfs: decayed, missing, and filled surfaces) and upper case letters (DMFT and DMFS) were used to indicate the permanent dentition. A brief pilot-tested questionnaire was given to the child at each foam treatment visit to evaluate his or her acceptance levels of the foam treatment. The

investigators recorded any side effects and complaints of the foam treatment at each foam treatment visit.

5.4. Saliva Sample Collections and Treatment

Stimulated saliva samples were collected at baseline before the foam treatment, at 1 month, 5 months, and 1 year. Saliva flow was stimulated by having the subject chew on a 2x2 cm square of paraffin (Parafilm "M" Laboratory Film, Pechiney Plastic Packaging, Chicago, IL). The participant was given 4 minutes to expectorate 2 ml of his or her saliva into a 15 ml sterile tube (Becton Dickinson, USA). The 2 ml of stimulated saliva was collected for microbiological assessment prior to any dental exam, cleaning or foam treatment and stored in the refrigerator immediately after collection prior to plating for microbiological assessment within 24 hours (see below).

At baseline, a thorough dental prophylaxis using a disposable prophylaxis cup and prophylaxis paste was performed on each subject after saliva sample collection. For participants with clinically detectable calculus, hand scalers were utilized to remove the calculus. After the dental prophylaxis, either F foam or PVPI-F foam (based on participant's randomization assignment) was placed into a disposable dual-arch fluoride tray (Oral-B Funtrays® by Oral B, USA). Either a

small or medium size tray was chosen depending on the size of the participant's mouth. The tray with a randomly assigned treatment foam type was placed into the participant's mouth for 4 minutes while the participant was holding a saliva ejector in between the upper and lower arches of the tray. Upon completion of treatment, the tray was removed and any excess foam inside the mouth was removed with a saliva ejector. The subject was instructed not to eat or drink anything for 30 minutes. The participant acceptance of the foam and any other complaints were reviewed immediately after the foam treatment.

5.5. Saliva Sample Processing, Microbiology, and Laboratory Procedures The collected and refrigerated saliva samples were chilled on ice during transportation to the microbiology laboratory. Dr. C. Hoover, the microbiologist responsible for plating saliva samples and quantifying bacterial content, was blinded to the subjects' treatment foam assignments (F or PVPI-F). All saliva samples were plated for MS, LB, and total viable counts (TVC) within 24 hours of collection. Each sample was sonicated for 10 seconds, diluted in PBS, and aliquots were removed and plated on Mitis Salivarius Sucrose Bacitricin (MSSB) agar for MS enumeration, on Rogosa Tomato Juice agar for LB enumeration and on BHI-blood agar for TVC enumeration. All plates were incubated in 85% N_2,

10% H_2, and 5% CO_2 for 48 to 96 hours before enumeration of bacterial colonies. The enumerations of MS, LB and TVC in saliva were calculated as colony forming units per ml of saliva (CFU/ml).

5.6. Statistical Analysis

The dmfs/DMFS increment and percentage of the subjects with new (incident) decay were calculated in both groups. The difference in dmfs/DMFS increments between the 2 groups was analyzed by the 2 sample Student's t-test. The percentage of the subjects with new decay was analyzed using the Chi-square test.

The colonization levels of the bacteria were determined for each subject for each time point: at baseline, 1 month, 6 months, and 1 year. The values obtained were converted to $\log_{10}$ (CFU/ml + 1) values ($\log_{10}$MS, $\log_{10}$LB, and $\log_{10}$TVC) so that parametric statistical tests could be applied to the data. Regression analyses were used to test differences while adjusting for covariates such as age and gender. For each subject, the statistically significant changes in $\log_{10}$MS and $\log_{10}$LB, if any, were calculated by comparing pre-treatment logarithm-transformed bacterial counts with those at 6 months and 1 year to

determine if the repeated PVPI-F treatment had a long-term impact on cariogenic bacteria re-colonization.

Baseline data (age, dmfs/DMFS, snacking frequencies and microbiology) between Group A and Group B were summarized with means and standard deviations (SDs); gender and racial/ethnic distributions of the 2 groups were summarized with percents. Pearson correlations were estimated between the MS, LB and TVC levels at 6 months and 1 year and each one of the following: snacking frequencies, snacking patterns, dmfs/DMFS, ds/DS, caries condition, and age.

Dr. Stuart Gansky was responsible for overseeing the study design, data management, generating data analysis plan and performing multifactor regression analysis. Dr. Ling Zhan was responsible for data entry and simple data analysis under Dr. Gansky's guidance.

6. Results

6.1. Subjects at Baseline

A total of 60 children (36 males and 24 females) were enrolled in the study with 31 subjects in the F group (control) and 29 subjects in the PVPI-F group. There were 6 families with 2 siblings enrolled in the study and 3 families with 3 siblings enrolled in this study. Randomization was at the child level (not at the family level). One family of 2 children was in the F group, 2 families of 2 children were in the PVPI-F group and 3 families of 2 children had 1 child in each group. Two families with 3 children enrolled had 2 children in the F group and 1 child in the PVPI-F group; the other family of 3 children had 1 child in the F group and 2 children in the PVPI-F group.

At baseline, the mean age of the subjects was 7.3 years (range: 6 to 9) in the F group and 7.4 years (range: 6 to 9) in the PVPI-F group (Table 1). The vast majority of the participants were Hispanic (Mexican, South American, or Cental American).

All subjects enrolled in the study were confirmed to have or have had active dental decay within the past year. The mean dmfs/DMFS values are given in Table 1. Caries indices (dmfs/DMFS and ds/DS) were similar between

the 2 groups at baseline. There was no relation of age and gender to any microbiological measurements and decay increments; bacterial counts were similar between the 2 groups at baseline.

Table 1 shows the subject parameters at baseline.

Table 1: Subject Parameters at Baseline

	F (n=31)	PVPI-F (n=29)
Mean (SD)* Age in Years	7.3 (1.1)	7.4 (1.0)
Gender Male:Female	16:15	20:9
Baseline Mean (SD)* dmfs/DMFS	20.2 (14.1)	19.7 (13.5)
Baseline Mean (SD)* ds/DS	3.4 (4.0)	4.2 (4.5)
Ethnicity (%)		
Hispanic	15 (48)	20 (69)
Middle Eastern	8 (26)	3 (10)
African American	4 (13)	1 (3)
Asian/Filipino	3 (10)	3 (10)
Caucasian	0 (0)	1 (3)
Others	1 (3)	1 (3)

*SD denotes standard deviation

6.2. Familial Aggregration

The percentage of variation due to families was calculated. The percent variation was calculated for the following variables: dmfs/DMFS, ds/DS, log_{10}MS, log_{10}LB and log_{10}TVC. The intra-family (intra-class) correlations were 0.60 for

dmfs/DMFS, 0.81 for ds/DS, 0.60 for $\log_{10}$MS, 0.50 for $\log_{10}$LB, and 0 for $\log_{10}$TVC.

6.3. Questionnaire Data

At the time of enrollment, each parent was required to fill out a questionnaire, which asked about the snacking frequency and oral hygiene habits and routines of the child. Snacking frequency did not correlate to microbiological levels or to the number of decayed surfaces ($-0.17 < r_s < 0.12$ Spearman's rank correlation test, $p>0.22$). All parents except 6 stated that their children were using fluoridated toothpaste. Three parents answered that they do not know if their children use fluoridated toothpaste; one of them belonged to the F group, and the other two to the PVPI-F group. Three other parents answered that their children do not use fluoridated toothpaste but that they use Colgate toothpaste (which is actually fluoridated); all of these participants belonged to the PVPI-F group.

6.4. Subjects at Completion

All but 5 subjects in the F group completed all of the 8 foam treatments. One participant dropped out of the study after the initial appointment, and 4 participants dropped out of the study after the first round of foam treatments.

However, there was one participant who completed all of the 8 foam treatments but did not return for the final saliva sample collection/examination. Also, it should be noted that the S3 saliva samples for 2 participants in the F group were misplaced and could not be retrieved.

All but 2 subjects in the PVPI-F group completed all of the 8 foam treatments. Both participants dropped out of the study after the first round of foam treatments.

Five subjects in the PVPI-F group complained of an unpleasant taste of the foam compared to 3 subjects in the F group. However, all subjects who complained about the taste of the treatment foam were able to complete the 4-minute foam treatment and graded their experience as tolerable. No long-term complaints or adverse effects were noted in either group.

6.5. Microbiological Data: at Baseline

The bacterial counts were converted into $\log_{10}$ (CFU/ml + 1). At baseline, the MS levels of 18 participants in the F group (58%) and 19 participants in the PVPI-F group (66%) exceeded 10^5 CFU/ml. Four participants, 2 in the F group and 2 in the PVPI-F group, did not have detectable levels of MS. All participants

except 13 (9 in the F group and 4 in the PVPI-F group) or approximately 78% percent of the participants had detectable levels of LB.

No significant correlation was found between bacterial levels and the subjects' ds (decayed surfaces) or ss-ds (smooth surfaces – decayed surfaces) scores ($-0.24 < r < 0.20$, $p>0.05$). No significant correlation was found between snacking pattern and microbiology measures ($r=0.02$ for $\log_{10}$MS, $r=0.21$ for $\log_{10}$LB, $r=-0.09$ for $\log_{10}$TVC, Spearman's rank correlation test, $p>0.05$). MS, LB, and TVC levels at baseline were similar between the 2 groups. A Pearson's correlation coefficient showed that the $\log_{10}$MS count at baseline positively correlated to the $\log_{10}$LB count ($r=0.46$, $p<0.01$).

6.6. Microbiological Data: Changes at 6 Months and 1 Year

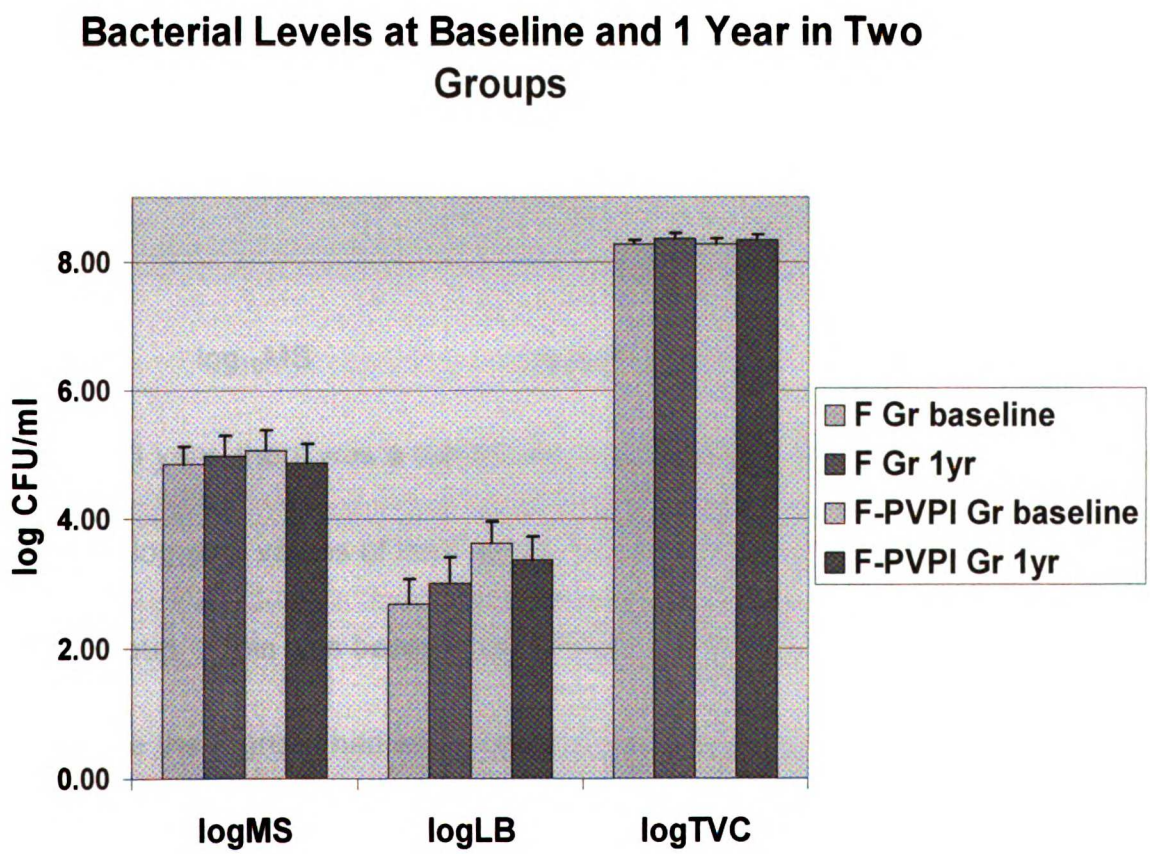
We compared the bacterial measurements at baseline to those at 6 months and 1 year. Table 2 shows the mean levels of $\log_{10}$MS, $\log_{10}$LB, and $\log_{10}$TVC at baseline, 6 months, and 1 year calculated for all subjects who completed their 1 year follow-up visits except the 1 subject who failed to have his cavities restored within 6 months. Figure 1 depicts the mean levels at baseline and 1 year only.

Table 2: Mean (SD)* $\log_{10}$ Bacterial Counts at Baseline, 6 Months, and 1 Year

	F Baseline (n=26)	F 6 Mo (n=26)	F 1 Yr (n=27)	PVPI-F Baseline (n=29)	PVPI-F 6 Mo (n=27)	PVPI-F 1 Yr (n=27)
$\log_{10}$MS	4.8 (1.6)	4.7 (1.7)	5.0 (1.7)	5.1 (1.6)	4.9 (1.7)	4.9 (1.6)
$\log_{10}$LB	2.5 (2.1)	3.1 (2.1)	3.0 (2.3)	3.6 (1.9)	3.2 (2.0)	3.4 (2.1)
$\log_{10}$TVC	8.3 (0.4)	8.2 (0.4)	8.4 (0.4)	8.3 (0.4)	8.3 (0.4)	8.3 (0.5)

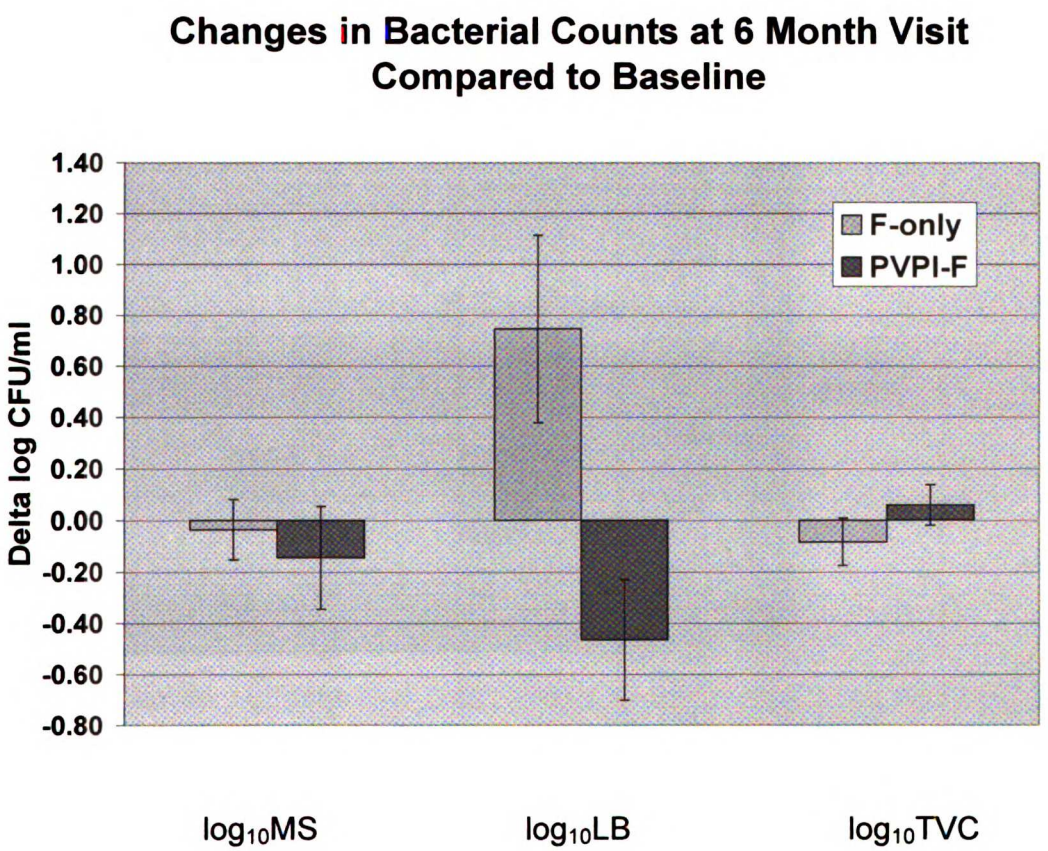
*SD denotes standard deviation.

Figure 1:



At 6 month, there was a significant difference in change of log_{10}LB from baseline between the 2 groups (Student t-test, p=0.03); however, no statistically significant changes were found on log_{10}MS and log_{10}TVC (Figure 2).

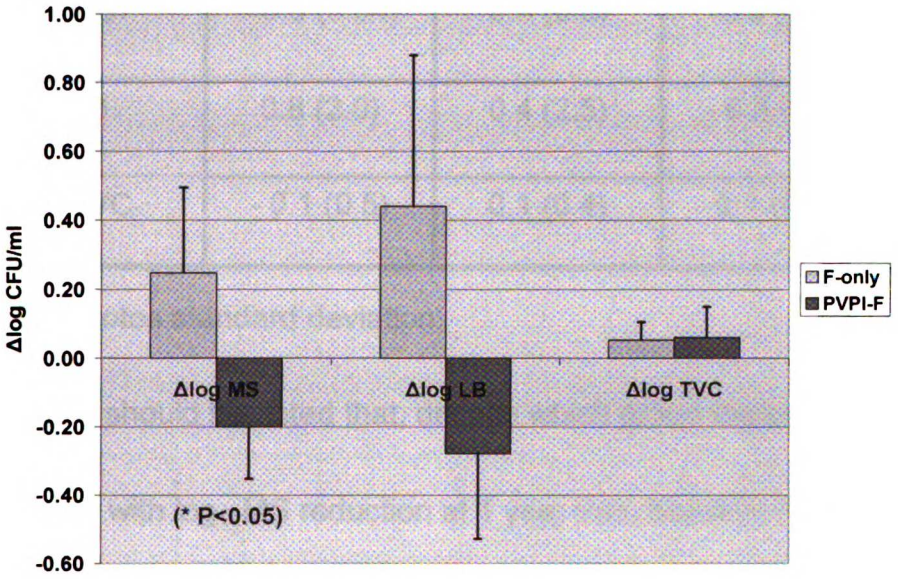
Figure 2:



At 1 year, there was a statistically significant difference between the change of log_{10}MS values of the F group from baseline to 1 year and that of the PVPI-F group. This was because the PVPI-F group had a reduction in log_{10}MS levels while the F group had an increase in log_{10}MS levels (Figure 3). However,

this does not imply any clinical significance because the change in the log value was less than 1. Moreover, there were no statistically significant changes in log_{10}LB and log_{10}TVC found between the 2 groups (+/- 95% CI; Student t-test, p= 0.33 and 0.92, respectively, assuming equal variance).

Figure 3: Cariogenic Bacterial Change at 1 year in F and PVPI-F Groups Compared to Baseline



*Error bars indicate standard errors, and 0.00 indicates baseline.

The mean (SD) changes in log_{10}MS, log_{10}LB and log_{10}TVC at 6 months and 1 year are listed in Table 3. There were no statistically significant changes in log_{10}MS, log_{10}LB and log_{10}TVC levels found in any group at 6 months or at 1 year compared to those levels at baseline (Student t-test, p>0.05). There were

no statistically significant changes in log_{10}MS, log_{10}LB and log_{10}TVC levels in either group at 1 year compared to those levels at baseline ($p>0.05$).

Table 3: Mean (SD)* Change in Microbiological Data between Baseline & 6 Months and 1 Yr

	F 6 Mo (n= 26)	F 1 Yr (n=27)	PVPI-F 6 Mo (n= 27)	PVPI-F 1 Yr (n=27)
Δlog_{10}MS	- 0.0 (0.64)	0.3 (0.6)	-0.2 (1.1)	-0.2 (0.8)
Δlog_{10}LB	0.8 (2.0)	0.4 (2.5)	- 0.5 (1.3)	-0.3 (1.4)
Δlog_{10}TVC	- 0.1 (0.5)	0.1 (0.4)	0.1 (0.4)	0.1 (0.5)

*SD denotes standard deviation.

It should be noted that, despite which group the subjects were in, subjects with log_{10}MS reduction at 1 year from baseline had significantly less total new caries surfaces and interproximal caries surfaces at 1 year compared to those who had static or elevated levels of log_{10}MS at 1 year (Student t-test, $p<0.05$).

The log_{10}LB change failed to show a significant impact on development of new caries although reduction in log_{10}LB was associated with less new interproximal caries surfaces (Student t test, $p=0.08$). However, subjects with reduced log_{10}TVC at 1 year had significantly more new pit and fissure caries

surfaces (Student t-test, $p<0.05$) and less total new caries surfaces (Student t test, $p=0.07$)

6.7. dmfs/DMFS Data: at Baseline

The dmfs/DMFS data were collected from all 60 subjects at baseline. Dr. Ling Zhan performed clinical exams on the subjects by using an intraoral mirror and a dental explorer as well as bilateral bitewing and, when necessary, periapical radiographic films. The baseline dmfs and DMFS indices are enumerated in Tables 4, 5, and 6 below. Caries indices were similar between the 2 groups at baseline.

Table 4: Mean (SD) Caries Indices (Primary Teeth) Data at Baseline for All Patients in Each Group

	# of teeth	ds	ss-ds	fs	ss-fs	ms	d_1s
F Group (n=31)	12.5 (4.9)	2.3 (3.6)	1.4 (2.3)	13.32 (10.3)	7.9 (7.9)	3.0 (5.5)	0.4 (1.3)
PVPI-F Group(n=29)	11.8 (4.5)	3.0 (4.1)	1.9 (2.8)	11.2 (9.8)	6.4 (6.7)	3.6 (7.5)	0.3 (1.1)

*SD denotes standard deviation.

*d_1s denotes non-cavitated enamel caries in primary teeth.

Table 5: Mean (SD) Caries Indices (Permanent Teeth) Data at Baseline for All Patients in Each Group

	# of teeth	DS	SS-DS	FS	SS-FS	MS	D_1S
F Group (n=31)	9.8 (5.4)	1.1 (2.2)	0.0 (0.2)	0.5 (1.3)	0.0 (0.0)	0.0 (0.0)	0.2 (0.6)
PVPI-F Group(n=29)	10.5 (4.6)	1.2 (1.7)	0.1 (0.4)	0.6 (1.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

*SD denotes standard deviation.

*D_1S denotes non-cavitated enamel caries in permanent teeth.

Table 6: Mean (SD) Total dmfs/DMFS Data at Baseline for All Patients in Each Group

	# of teeth	ds/DS	ss-ds/DS	fs/FS	ss-fs/FS	ms/MS	ss-dfs/DFS	dmfs/DMFS
F Grp (n=31)	22.3 (1.8)	3.4 (4.0)	1.4 (2.3)	13.8 (10.2)	7.9 (7.9)	3.0 (5.5)	9.4 (8.6)	20.2 (14.0)
PVPI-F Grp (n=29)	22.2 (2.0)	4.2 (4.5)	2.0 (2.7)	11.8 (9.7)	6.4 (6.7)	3.6 (7.5)	8.4 (6.7)	19.7 (13.5)

6.8. DMFS Data: Changes at 1 Year from Baseline

The dmfs/DMFS data were collected from 27 participants from the F group and 27 participants from the PVPI-F group. Dr. Ling Zhan evaluated all the participants utilizing the same methodology she had used to collect the baseline dmfs/DMFS data. The mean changes in the dmfs/DMFS and the number of decayed surfaces from the baseline to 1 year are shown in Table 7.

One of the participants in the F group (PR#24) had issues with his dental insurance and was not able to get his carious teeth restored and presented with a worse dental condition at 1 year. Table 8 illustrates the dmfs/DMFS indices excluding this particpant's data.

Table 7: Mean (SD) Change in dmfs/DMFS and ds/DS at 1 Year

	New dmfs/DMFS	New ds/DS	New interproximal ss-ds/DS	New pit/fissure ds/DS	New BL ss-ds/DS	New total ss-ds/DS
F Group (n=27)	2.3 (3.1)	0.9 (1.7)	0.6 (0.8)	0.2 (0.7)	0.2 (0.8)	0.7 (1.3)
PVPI-F Grp(n=27)	5.2 (7.6)	0.8 (1.1)	0.4 (0.7)	0.3 (0.7)	0.1 (0.3)	0.5 (0.8)

Table 8: Mean (SD) Change in dmfs/DMFS and ds/DS at 1 Year Excluding PR24

	New dmfs/DMFS	New ds/DS	New interproximal ss-ds/DS	New pit/fissure ds/DS	New BL ss-ds/DS	New total ss-ds/DS
F Group (n=27)	2.0 (2.5)	0.6 (1.2)	0.5 (0.8)	0.1 (0.6)	0.0 (0.2)	0.5 (0.8)
PVPI-F Grp (n=27)	5.2 (7.7)	0.7 (0.9)	0.3 (0.6)	0.1 (0.3)	0.3 (0.7)	0.4 (0.7)

As shown in Table 9 below, 11 participants in the F group (41%) and 11 participants in the PVPI-F group (41%) presented with new carious lesions at 1

year. There was no statistically significant difference in 1 year caries incidence —new ds/DS, new ss-ds/DS or pits & fissure ds/DS—between the 2 groups in all subjects or all but PR24 who did not have dental treatment done during the course of the study (Student t-test, $p>0.05$).

Even though there were fewer participants with new smooth-surface decayed surfaces (ss-ds/DS) in the PVPI-P group than in the F group—30% and 37%, respectively—there was no statistically significant difference. The percentage of participants with new interproximal caries in the F group was almost double of that in the PVPI-F group—43% and 23%, respectively; however, this was not a statistically significant difference (Pearson Chi-square test, $p>0.05$). The PVPI-F group had significantly more new dmfs/DMFS than the F group (Student t-test, $p<0.05$), but it is due to the higher number of decayed surfaces in the PVPI-F group than in the F group at baseline.

Table 9: Number of Participants with New ds/DS, ss-ds/DS, and interproximal ds/DS at 1 Year

	New ds/DS	New total ss-ds/DS	New interproximal ss-ds/DS
F Group (n=27)	11 (41%)	10 (37%)	10 (37%)
PVPI-F Grp(n=27)	11 (41%)	8 (30%)	6 (22%)

6.9. Correlation between Microbiological Data and DMFS Data

One interesting finding was that an increase in log_{10}LB was positively correlated with increased interproximal ds/DS (Pearson's correlation, r=0.40). It was also noted that an increase in log_{10}TVC was negatively correlated with new pits and fissure ds/DS (Pearson's correlation, r=-0.46). No other significant correlations were observed between new ds/DS, interproximal ds/DS, new ss-ds/DS and new pit and fissure ds/DS and log_{10}MS, log_{10}LB and log_{10}TVC at 1 year.

7. Discussion

Dental caries is an infectious and transmissible disease that is characterized by an overwhelming bacterial challenge by MS and LB. The current standard of care usually involves surgical removal and restoration of carious tooth structure, application of topical fluoride, oral hygiene, and dietary counseling[38,56]. This traditional approach does not address caries, which is a bacteria-mediated disease, in a comprehensive manner[57,58]. This study was conducted to contribute to the concerted efforts of many to identify and evaluate antimicrobial agents and regimens that can reduce bacterial challenge in individuals with high caries risk.

Previous studies have supported the possible usefulness of iodine in caries prevention. In 1979, Caufield *et al.* showed that after 3 topical applications of 2% iodine-potassium iodide solution (I_2-KI), MS levels in plaque and saliva could be reduced for 20-24 weeks after treatment[53]. Other studies demonstrated that either I_2-KI solution or iodine could decrease detectable levels of *S. mutans* and LB in the plaque of occlusal fissure caries by 98%[59,60].

A previous study by Zhan *et al.* consisted of 1 single application of 10% PVPI solution, applied by swabbing, in 2- to 5-year-old children with severe

caries under general anesthesia (GA) immediately following full-mouth rehabilitation. Saliva samples were collected at baseline and after 1 hour, 3 weeks, and 3 months after treatment. They found mean MS and LB levels were significantly suppressed at all follow-up visits in the PVPI group up to 3 months. However, the single PVPI treatment did not reduce the formation of new carious lesions at 1 year[55].

Berkowitz *et al.* have found similar results: a significant reduction in MS levels in children with early childhood caries following PVPI treatment in the operating room (OR). They recruited 2- to 5-year-olds undergoing dental treatment under GA. A 0.2 ml solution of 10% PVPI was applied to the teeth immediately following full-mouth rehabilitation. The 10% PVPI solution had a significant suppressive effect on salivary MS levels in these participants with ECC[61].

In the present study, the blueberry flavor in the PVPI-F foam was used to improve clinical acceptance for the study participants. The formulation was well accepted by the majority of the participants except 5 subjects in the PVPI-F group and 3 subjects in the F, who complained of an unpleasant taste of the foam. However, all participants graded both foams as tolerable.

In the present study, no significant reductions in MS and LB levels were observed at 1 year in PVPI-F group compared to those at baseline. The foam treatment was not as successful in reducing oral cariogenic bacterial levels as the swabbing method using a solution in the OR as in the studies of Zhan *et al.*[55] and Berkowitz *et al.*[61].

The following factors may have contributed to the limited efficacy of the PVPI-F foam regimen in the present study.

1) The cariogenic oral bacteria, namely MS and LB, may be more susceptible to the iodine in the GA environment. In the Berkowitz *et al.* and Zhan *et al.* studies, PVPI was applied by swabbing a solution on the teeth of participants under GA in a well-controlled environment. Due to the medication used for GA, their participants' mouths remained dry for a long time, probably allowing a lengthy retention time for the PVPI. It is also possible that due to the dry conditions in the mouth, the bacteria may have been desiccated and more susceptible to PVPI.

On the other hand, in the present study, there was much less control over the oral environment because it was conducted in the regular dental clinic setting. The participants' saliva may have diluted PVPI or interfered with its attachment to

oral structures and ultimately decreasing its efficacy. Iodine also may have bound to the various proteins in the saliva and may not have been easily available to interact with the oral cariogenic bacteria.

The participants in the Berkowitz *et al.* and Zhan *et al.* studies had full-mouth rehabilitation prior to PVPI treatment. Even though research has historically supported that dental restorations alone do not have any significant effect on the overall bacterial loading in the remainder of the mouth, a recent study by Amin *et al.* has demonstrated that extensive one-time restorative dental treatment results in a significant suppression of *S. mutans* levels at 6 months following dental treatment[51].

2) In the present study, all the restorative needs of the subjects were not met at once in the OR. Rather, the participants received the necessary restorative needs within 6 months after their baseline exams with the exception of 1 participant in the F group, whose parents encountered issues with their dental insurance company and could not get treatment for the child in a timely manner.

3) The delivery vehicle was different for this study. In the Berkowitz and Zhan studies, 10% PVPI solution containing 1% active iodine was used whereas

in this present study, PVPI foam with 1% active iodine was utilized. Although the PVPI foam contained the stated amount of iodine, we cannot know whether the foam allowed the PVPI to be readily released into the dental plaque of the participants in comparison to the solution used in prior studies. We chose the foam vehicle because we found it to be clinically acceptable to children since the foam vehicle is routinely used for fluoride applications in pediatric dentistry.

Despite the numerous and frequent F or PVPI-F treatments applied in the present study, 11 subjects in the F group (41%) and 11 subjects in the PVPI-F group (41%) presented with new carious lesions at 1 year. This clearly illustrates how difficult it is to control caries in high caries risk individuals even with high concentration fluoride treatment on multiple occasions.

It was also interesting to note that an increase in $\log_{10}$LB was positively correlated with increased interproximal ds/DS (Pearson's correlation, r=0.40). Subjects with reduced $\log_{10}$MS showed significantly less new total caries surfaces and interproximal caries surfaces. These results support the notion that reduced cariogenic bacterial levels will lead to reduction of development of new carious lesions in high caries risk children. It is also interesting to find that subjects with reduced $\log_{10}$TVC showed more new pits and fissure ds/DS and

close-to-statistically-significant less number of total new caries surfaces. This may be indicative of some mechanism of ecologic balance of the oral flora on caries etiology. More studies are needed on developing a feasible antimicrobial regimen against cariogenic bacteria in children with high caries risk.

8. Conclusion

In this randomized double-blinded study, PVPI-F foam was ineffective in significantly reducing cariogenic bacterial counts in 6- to 9-year-old children with active caries. In contrast to some studies that previously reported significant bacterial reductions by PVPI solution in younger children, in the present study, MS and LB levels were not significantly reduced in the PVPI-F foam group compared to the F control group. This may be due to the different application conditions and vehicles. Despite multiple applications of F or PVPI-F over a period of 12 months, neither the bacterial counts nor new dmfs/DMFS decreased significantly. Further studies are necessary to evaluate whether the promising results for PVPI reported in prior studies can be realized with different delivery conditions, vehicles or regimens.

9. References

1. Featherstone JD. The science and practice of caries prevention. J Am De nt Assoc 2000;131(7):887-99.
2. Mouradian WE, Wehr E, Crall JJ. Disparities in children's oral health and access to dental care. JAMA 2000;284(20):2625-31.
3. National Institute of Dental Research, Epidemiology and Oral Disease Prevention Program. Oral health of United States Children: The A National S urvey of Dental Caries in U.S. School Children, 1986-1987—national and regional findings. Bethesda, Md.: U.S. Department of Health and Human Services, Public Health Service, National Institutesof Health; 1989, NIH publication 89-2247.
4. Macek MD, Heller KE, Selwitz RH, Manz MC. Is 75 percent of dental caries really found in 25 percent of the population? J Public Health Dent 2004;64(1):20-5
5. Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, Eke PI, Beltrán-Aguilar ED, Horowitz AM, Li CH. Trends in oral health status: United States, 1988-1994 and 1999-2004. National Center for HealthS tatistics, Centers for Disease Control and Prevention, U.S. Department of He alth and Human Services, Hyattsville, Maryland 20782, USA.
6. Kaste LM, Selwitz RH, Oldakowski RJ, Brunelle JA, Winn DM, Brown LJ. Coronal caries in the primary and permanent dentition of children and adolescents 1-17 years of age: United States, 1988-1991. J Dent Res 1996;75 Spec No:631-41.
7. Jenkins GN. Recent changes in dental caries. Br Med J (Clin Res Ed) 19 85;291(6505):1297-8.
8. Hargreaves JA, Thompson GW, Wagg BJ. Changes in caries prevalence of Isle of Lewis children between 1971 and 1981. Caries Res 1983;17(6):554-9.
9. Featherstone JD. The caries balance. Feb 2003 CDA Journal 31(2):129
10. Pollick HF. Report of the California oral health needs assessment of children, 1993-1994. The Dental Health Foundation, Oakland, CA 1999.
11. Ramos-Gomez FJ, Shepard DS. Cost-effectiveness model for prevention of early childhood caries. J Calif Dent Assoc 1999;27(7):539-44.

12. Nuttall NM, Steele JG, Pine CM, White D and Pitts NB. The impact of oral health on people in the UK in 1998. British Dental Journal 2001 Feb;190(3):121-126.
13. Ismail AI. Determinants of health in children and the problem of early childhood caries. Pediatr Dent 2003;25(4):328-33.
14. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50(4):353-80.
15. Wan A, Seow W, Purdie D, Bird P, Walsh L, and Tudehope D. Oral colonization of Streptococcus mutans in six-month-old predentate infants. J Dent Res 2001 Dec;80(12):2060-5.
16. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50(4):353-80.
17. Wan A, Seow W, Purdie D, Bird P, Walsh L, and Tudehope D. Oral colonization of Streptococcus mutans in six-month-old predentate infants. J Dent Res 2001 Dec;80(12):2060-5.
18. van Houte, J. 1994. Role of micro-organisms in caries etiology. J Dent Res 73:672-681.
19. Teanpaisan R, Thitasomakul S, Piwat S, Thearmontree A, Pithpornchaiyakul W, Chankanka O. Longitudinal study of the presence of mutans streptococci and lactobacilli in relation to dental caries development in 3-24 month old Thai children. Int Dent J. 2007 Dec;57(6):445-51.
20. Nomura Y, Senpuku H, Hanada N, Kumagai T. Mutans streptococci and Lactobacillus as risk factors for dental caries in 12-year-old children. Jpn J Infect Dis 2001;54(1):43-5.
21. Campus G, Lumbau A, Bachisio SL. Caries experience and streptococci and lactobacilli salivary levels in 6-8-year-old Sardinians. Int J Paediatr Dent 2000;10(4):306-12.
22. Farsi N. Dental caries in relation to salivary factors in Saudi population groups.
J Contemp Dent Pract. 2008 Mar 1;9(3):16-23.
23. Kohler B and Bratthall D. Practical method to facilitate estimation of Streptococcus mutans levelsin saliva. J of Clinical Microbiology 1979 May;9(5): 584-588.
24. Ramos-Gomez FJ, Weintraub JA, Gansky SA, Hoover CI, Featherstone JD. Bacterial, behavioral and environmental factors associated with early childhood

caries. J Clin Pediatr Dent 2002;26(2):165-73.
25. Kohler B, Bratthall D. Intrafamilial levels of Streptococcus mutans and some aspects of the bacterial transmission. Scand J Dent Res 1978;86(1):35-42.
26. Keene JH. Sampling of cariogenic microorganisms in human populations. Oral Microbiol Immunol 1986:1:7-12.
27. Mundorff S, Eisenberg A, Leverett D, Espeland M, and Proskin H. Correlations between numbers of microflora in plaque and saliva. Caries Res 1990;24;312-317.
28. Declerck D, Leroy R, Martens L, Lesaffre E, Garcia-Zattera MJ, Vanden Broucke S, Debyser M, Hoppenbrouwers K. Factors associated with prevalence and severity of caries experience in preschool children. Community Dent Oral Epidemiol. 2008 Apr;36(2):168-78.
29. van Houte J. Microbiological predictors of caries risk. Adv Dent Res 1993 Aug;7(2):87-96.
30. Milgrom P and Weinstein, P. Early childhood caries: A team approach to prevention and treatment. Seattle, WA: University of Washington; 1999.
31. Kohler B, et al. Preventive measures in mothers influence the establishment of Streptococcus mutans in their infants. Arch Oral Biol 1983;23:225-231.
32. Van Loveren C, Buijs JF, and ten Cate JM. Similarity of bacteriocin activity profiles of mutans streptococci within the family when the children acquire the strains after the age of 5. Caries Res 2000;34:481-485.
33. Mattos-Graner R, Li Y, et al. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. J Clin Microbiol 2001;39:13-18.
34. Mandel ID. The functions of saliva. J Dent Res 1987;66 Spec No:623-7.
35. Holloway, P.J. (1983). The role of sugar in the etiology of dental caries. Journal of Dentistry 11:189-213.
36. Arcella D, Ottolenghi L, Polimeni A and Leclercq C. The relationship between frequency of carbohydrates intake and dental caries: a cross-sectional study in Italian teenagers. Public Health Nutr 2002 Aug;5(4):553-60.
37. Wright JT, Cutter GR, Dasanayake AP, Stiles HM, Caufield PW. Effect of conventional dental restorative treatment on bacteria in saliva. Community Dent Oral Epidemiol 1992;20(3):138-43.
38. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol 1999;27(1):31-40.
39. Featherstone JD. The continuum of dental caries--evidence for a dynamic

disease process. J Dent Res 2004;83 Spec No C:C39-42.
40. Lynch H, Milgrom P. Xylitol and dental caries: an overview for clinicians. J Calif Dent Assoc 2003;31(3):205-9.
41. 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.
42. Hrimech M, Mayrand D, Grenier D, Trahan L. Xylitol disturbs protein synthesis, including the expression of HSP-70 and HSP-60, in *Streptococcus mutans.* Oral Microbiol Immunol 2000;15(4):249-57.
43. Miake Y, Saeki Y, Takahashi M, Yanagisawa T. Remineralization effects of xylitol on demineralized enamel. J Electron Microsc (Tokyo) 2003;52(5):471-6.
44. Alanen P. Does chewing explain the caries-preventive results with xylitol? J Dent Res 2001;80(7):1600-1.
45. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 1994;73(3):682-91.
46. Kidd EA. Role of chlorhexidine in the management of dental caries. Int Dent J 1991;41(5):279-86.
47. van Lunsen DM, de Soet JJ, Weerheijm KL, Groen HJ, Veerkamp JS. Effects of dental treatment and single application of a 40% chlorhexidine varnish on mutans Streptococci in young children under intravenous anaesthesia. Caries Res 2000;34(3):268-74.
48. LeVeen HH, LeVeen RF, LeVeen EG. The mythology of povidone-iodine and the development of self-sterilizing plastics. Surg Gynecol Obstet 1993;176(2):183-90.
49. Zamora JL. Chemical and microbiologic characteristics and toxicity of povidone-iodine solutions. Am J Surg 1986;151(3):400-6.
50. Khan MN, Naqvi AH. Antiseptics, iodine, povidone iodine and traumatic wound cleansing. J Tissue Viability 2006;16(4):6-10.
51. Amin MS, Harrison RL, Benton TS, Roberts M, Weinstein P. Effect of povidone-iodine on Streptococcus mutans in children with extensive dental caries.
52. Gibbons RJ, Depaola PF, Spinell DM, Skobe Z. Interdental localization of Streptococcus mutans as related to dental caries experience. Infect Immun 1974;9(3):481-8.
53. Caufield PW, Gibbons RJ. Suppression of Streptococcus mutans in the mouths of humans by a dental prophylaxis and topically-applied iodine. J Dent Res 1979;58(4):1317-26.

54. Lopez L, Berkowitz R, Spiekerman C, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries: a follow-up report. Pediatr Dent 2002;24(3):204-6.
55. Zhan L, Featherstone JD, Gansky SA, Hoover CI, Fujino T, Berkowitz RJ, et al. Antibacterial treatment needed for severe early childhood caries. J Public Health Dent 2006;66(3):174-9.
56. Featherstone JD. Caries prevention and reversal based on the caries balance. Pediatr Dent 2006;28(2):128-32; discussion 92-8.
57. Ali YA, Chandranee NJ, Wadher BJ, Khan A, Khan ZH. Relationship between caries status, colony forming units (cfu) of Streptococcus mutans and Snyder caries activity test. J Indian Soc Pedod Prev Dent 1998;16(2):56-60.
58. Crall JJ. Rethinking prevention. Pediatr Dent 2006;28(2):96-101; discussion 92-8.
59. Schaeken MJ, de Jong MH, Franken HC, van der Hoeven JS. Effect of chlorhexidine and iodine on the composition of the human dental plaque flora. Caries Res 1984;18(5):401-7.
60. Meiers JC, Schachtele CF. The effect of an antibacterial solution on the microflora of human incipient fissure caries. J Dent Res 1984;63(1):47-51.
61. Berkowitz R J HK, M McDermott, MT Whelehan, P Ragusa, RJ Billings. Chemotherapeutic Suppression of MS Levels in Children Treated for Severe ECC. Paper presented at: IADR 85th General Session, 2007; New Orleans, Louisiana.

10. Appendix A: Data Spreadsheets

F Group: Microbiology Raw Data

	S1			S2		
ID	MS	LB	TVC	MS	LB	TVC
PR01	40,000	8,000	100,000,000	3,330	1,620	60,000,000
PR02	326,000	400,000	170,000,000	99,000	38,000	110,000,000
PR05	700,000	400,000	260,000,000	1,100,000	230,000	410,000,000
PR06	76,000	330	370,000,000	290,000	1,250	690,000,000
PR11	500,000	0	130,000,000	243,000	60	130,000,000
PR12	12,000	0	320,000,000	26,000	0	470,000,000
PR13	59,000	0	270,000,000	7,700	0	670,000,000
PR14	104,000	240	90,000,000	35,000	0	40,000,000
PR15	800,000	10	310,000,000	800,000	420	210,000,000
PR17	34,000	0	380,000,000	700,000	0	530,000,000
PR 18	78,000	0	120,000,000	92,000	10	130,000,000
PR 21	3,500,000	58,000	800,000,000	N/A	N/A	N/A
PR 24	2,600,000	2,100,000	440,000,000	800,000	700,000	260,000,000
PR 27	119,000	0	140,000,000	73,000	0	90,000,000
PR 28	0	0	230,000,000	0	380	110,000,000
PR 32	0	0	110,000,000	0	0	40,000,000
PR 35	1,800,000	43,000	190,000,000	600,000	7,000	360,000,000
PR 37	59,000	12,000	20,000,000	3,000	260	10,000,000
PR38	1,400,000	28,000	1,230,000,000	700,000	120,000	60,000,000
PR40	6,000	330	250,000,000	13,000	1,020	130,000,000
PR 42	76,000	60	1,100,000,000	46,000	23,000	180,000,000
PR 44	3,400,000	500,000	360,000,000	110,000	3,000	140,000,000
PR45	1,000,000	199,000	380,000,000	400,000	20,000	100,000,000
PR46	600,000	210	230,000,000	1,000,000	181,000	250,000,000
PR48	19,000	9,000	90,000,000	3,000	360	80,000,000
PR53	140,000	990	130,000,000	291,000	0	150,000,000
PR54	18,000	950	70,000,000	152,000	5,000	80,000,000
PR55	2,000,000	113,000	40,000,000	1,900,000	76,000	80,000,000
PR57	293,000	20	280,000,000	1,000,000	0	120,000,000
PR58	1,000,000	21,000	240,000,000	102,000	75,000	140,000,000
PR59	2,000,000	0	330,000,000	800,000	0	80,000,000

	S3			S4		
ID	MS	LB	TVC	MS	LB	TVC
PR01	12,000	12,000	580,000,000	800,000	125,000	510,000,000
PR02	118,000	700,000	700,000,000	1,300,000	104,000	690,000,000
PR05	600,000	400,000	260,000,000	400,000	800,000	100,000,000
PR06	400,000	0	730,000,000	500,000	76,000	540,000,000
PR11	1,200,000	0	520,000,000	134,000	0	220,000,000
PR12	1,100,000	15,000	220,000,000	310,000	28,000	870,000,000
PR13	31,000	0	180,000,000	56,000	70	140,000,000
PR14	119,000	200	120,000,000	900,000	34,000	210,000,000
PR15	3,700,000	40,000	160,000,000	N/A	N/A	N/A
PR17	68,000	60	70,000,000	43,000	0	90,000,000
PR 18	116,000	760	60,000,000	128,000	220,000	250,000,000
PR 21	N/A	N/A	N/A	N/A	N/A	N/A
PR 24	1,000,000	5,000,000	120,000,000	2,700,000	300,000	220,000,000
PR 27	55,000	760	60,000,000	47,000	0	370,000,000
PR 28	0	470	210,000,000	0	590	110,000,000
PR 32	0	0	120,000,000	0	0	90,000,000
PR 35	286,000	160	940,000,000	500,000	400	160,000,000
PR 37	46,000	14,000	20,000,000	37,000	11,000	50,000,000
PR38	300,000	400,000	130,000,000	3,800,000	600,000	3,170,000,000
PR40	260	550	50,000,000	128,000	10	130,000,000
PR 42	124,000	376,000	110,000,000	116,000	700,000	120,000,000
PR 44	n/a	n/a	n/a	4,200,000	860	350,000,000
PR45	n/a	n/a	n/a	n/a	n/a	n/a
PR46	317,000	70,000	640,000,000	500,000	0	550,000,000
PR48	18,000	430	60,000,000	48,000	20	90,000,000
PR53	139,000	0	130,000,000	117,000	0	180,000,000
PR54	109,000	12,000	90,000,000	600,000	350	200,000,000
PR55	3,300,000	6,000	260,000,000	2,500,000	27,000	690,000,000
PR57	n/a	n/a	n/a	155,000	5000	300,000,000
PR58	800,000	114,000	270,000,000	600,000	0	10,000,000
PR59	n/a	n/a	n/a	n/a	n/a	n/a

PVPI-F Group: Microbiology Raw Data

	S1			S2		
ID	MS	LB	TVC	MS	LB	TVC
PR03	2,400,000	800,000	490,000,000	135,000	137,000	200,000,000
PR04	419,000	116,000	340,000,000	1,100,000	6,000	140,000,000
PR07	106,000	5,000	90,000,000	137,000	42,000	140,000,000
PR08	1,100,000	1,200,000	480,000,000	27,000	110	190,000,000
PR09	1,300,000	42,000	410,000,000	700,000	34,000	310,000,000
PR10	73,000	2,910	80,000,000	359,000	0	290,000,000
PR16	270	600	10,000,000	1,980	620	510,000,000
PR19	123,000	111,000	180,000,000	2,400,000	337,000	380,000,000
PR20	1,500,000	23,000	380,000,000	700,000	40	310,000,000
PR22	2,100,000	73,000	240,000,000	1,700,000	75,000	190,000,000
PR23	186,000	82,000	200,000,000	196,000	32,000	90,000,000
PR25	800,000	119,000	120,000,000	395,000	99,000	10,000,000
PR26	29,000	910	270,000,000	171,000	1,130	180,000,000
PR29	0	0	250,000,000	0	0	90,000,000
PR30	0	1,450	310,000,000	0	390	90,000,000
PR31	43,000	271,000	560,000,000	2,170,000	600,000	590,000,000
PR33	19,000	3,000	130,000,000	50,000	15,000	140,000,000
PR34	108,000	64,000	60,000,000	1,400,000	294,000	620,000,000
PR36	12,000	0	110,000,000	25,000	170	140,000,000
PR39	400,000	71,000	10,000,000	337,000	63,000	130,000,000
PR41	5,500,000	7,000	850,000,000	4,300,000	15,000	1,260,000,000
PR43	2,000,000	1,140	420,000,000	11,000	0	150,000,000
PR47	88,000	920	70,000,000	151,000	19,000	200,000,000
PR49	7,000,000	239,000	160,000,000	154,000	88,000	760,000,000
PR50	284,000	10	830,000,000	201,000	70	1,060,000,000
PR51	500,000	0	110,000,000	3,000,000	60	800,000,000
PR52	42,000	0	40,000,000	9,000	0	20,000,000
PR56	296,000	4,000	160,000,000	82,000	200	110,000,000
PR60	3,700,000	20,000	750,000,000	500,000	700	410,000,000

	S3			S4		
ID	MS	LB	TVC	MS	LB	TVC
PR03	2,700,000	1,200,000	980,000,000	2,300,000	1,100,000	1,290,000,000
PR04	285,000	270	210,000,000	372,000	13,000	350,000,000
PR07	72,000	7,000	230,000,000	59,000	10,000	230,000,000
PR08	112,000	400,000	400,000,000	400,000	340	520,000,000
PR09	3,000,000	36,000	360,000,000	500,000	15,000	190,000,000
PR10	400,000	300	70,000,000	58,000	140	250,000,000
PR16	N/A	N/A	N/A	N/A	N/A	N/A
PR19	135,000	21,000	40,000,000	300,000	35,000	80,000,000
PR20	500,000	560	130,000,000	34,000	800	20,000,000
PR22	57,000	110	340,000,000	212,000	70	160,000,000
PR23	203,000	4,000	90,000,000	49,000	7,000	80,000,000
PR25	2,300,000	4,100,000	220,000,000	3,300,000	2,100,000	630,000,000
PR26	1,400,000	21,000	330,000,000	1,300,000	70,000	960,000,000
PR29	0	10	60,000,000	0	0	140,000,000
PR30	0	570	240,000,000	0	990	350,000,000
PR31	3,400,000	6,000	500,000,000	2,100,000	237,000	1,120,000,000
PR33	9,000	870	70,000,000	7,000	1,830	60,000,000
PR34	1,900,000	300,000	280,000,000	37,000	400,000	420,000,000
PR36	4,000	0	100,000,000	24,000	0	140,000,000
PR39	850	510	90,000,000	400,000	59,000	30,000,000
PR41	2,000,000	18,000	530,000,000	3,700,000	66,000	290,000,000
PR43	11,000	0	210,000,000	44,000	0	160,000,000
PR47	263,000	25,000	140,000,000	198,000	48,000	60,000,000
PR49	82,000	900,000	330,000,000	86,000	2,300,000	170,000,000
PR50	500,000	0	650,000,000	252,000	40	640,000,000
PR51	4,300,000	0	770,000,000	135,000	0	900,000,000
PR52	14,000	0	270,000,000	14,000	0	100,000,000
PR56	300,000	341,000	340,000,000	311,000	7,000	390,000,000
PR60	n/a	n/a	n/a	n/a	n/a	n/a

F Group: Log Values of Microbiology Data
Baseline (S1) and 2 Months (S2), 6 Months (S3), and 1 Year (S4)

	S1			S2		
ID	logMS	logLB	logTVC	logMS	logLB	logTVC
PR01	4.60	3.90	8.00	3.52	3.21	7.78
PR02	5.51	5.60	8.23	5.00	4.58	8.04
PR05	5.85	5.60	8.41	6.04	5.36	8.61
PR06	4.88	2.52	8.57	5.46	3.10	8.84
PR11	5.70	0.00	8.11	5.39	1.79	8.11
PR12	4.08	0.00	8.51	4.41	0.00	8.67
PR13	4.77	0.00	8.43	3.89	0.00	8.83
PR14	5.02	2.38	7.95	4.54	0.00	7.60
PR15	5.90	1.04	8.49	5.90	2.62	8.32
PR17	4.53	0.00	8.58	5.85	0.00	8.72
PR 18	4.89	0.00	8.08	4.96	1.04	8.11
PR 21	6.54	4.76	8.90	drop	drop	drop
PR 24	6.41	6.32	8.64	5.90	5.85	8.41
PR 27	5.08	0.00	8.15	4.86	0.00	7.95
PR 28	0.00	0.00	8.36	0.00	2.58	8.04
PR 32	0.00	0.00	8.04	0.00	0.00	7.60
PR 35	6.26	4.63	8.28	5.78	3.85	8.56
PR 37	4.77	4.08	7.30	3.48	2.42	7.00
PR38	6.15	4.45	9.09	5.85	5.08	7.78
PR40	3.78	2.52	8.40	4.11	3.01	8.11
PR 42	4.88	1.79	9.04	4.66	4.36	8.26
PR 44	6.53	5.70	8.56	5.04	3.48	8.15
PR45	6.00	5.30	8.58	5.60	4.30	8.00
PR46	5.78	2.32	8.36	6.00	5.26	8.40
PR48	4.28	3.95	7.95	3.48	2.56	7.90
PR53	5.15	3.00	8.11	5.46	0.00	8.18
PR54	4.26	2.98	7.85	5.18	3.70	7.90
PR55	6.30	5.05	7.60	6.28	4.88	7.90
PR57	5.47	1.32	8.45	6.00	0.00	8.08
PR58	6.00	4.32	8.38	5.01	4.88	8.15
PR59	6.30	0.00	8.52	5.90	0.00	7.90

	S3			S4		
ID	logMS	logLB	logTVC	logMS	logLB	logTVC
PR01	4.08	4.08	8.76	5.90	5.10	8.71
PR02	5.07	5.85	8.85	6.11	5.02	8.84
PR05	5.78	5.60	8.41	5.60	5.90	8.00
PR06	5.60	0.00	8.86	5.70	4.88	8.73
PR11	6.08	0.00	8.72	5.13	0.00	8.34
PR12	6.04	4.18	8.34	5.49	4.45	8.94
PR13	4.49	0.00	8.26	4.75	1.85	8.15
PR14	5.08	2.30	8.08	5.95	4.53	8.32
PR15	6.57	4.60	8.20	drop	drop	drop
PR17	4.83	1.79	7.85	4.63	0.00	7.95
PR 18	5.06	2.88	7.78	5.11	5.34	8.40
PR 21	drop	drop	drop	drop	drop	drop
PR 24	6.00	6.70	8.08	6.43	5.48	8.34
PR 27	4.74	2.88	7.78	4.67	0.00	8.57
PR 28	0.00	2.67	8.32	0.00	2.77	8.04
PR 32	0.00	0.00	8.08	0.00	0.00	7.95
PR 35	5.46	2.21	8.97	5.70	2.60	8.20
PR 37	4.66	4.15	7.30	4.57	4.04	7.70
PR38	5.48	5.60	8.11	6.58	5.78	9.50
PR40	2.42	2.74	7.70	5.11	1.04	8.11
PR 42	5.09	5.58	8.04	5.06	5.85	8.08
PR 44	n/a	n/a	n/a	6.62	2.94	8.54
PR45	n/a	n/a	n/a	DROP	DROP	DROP
PR46	5.50	4.85	8.81	5.70	0.00	8.74
PR48	4.26	2.63	7.78	4.68	1.32	7.95
PR53	5.14	0.00	8.11	5.07	0.00	8.26
PR54	5.04	4.08	7.95	5.78	2.55	8.30
PR55	6.52	3.78	8.41	6.40	4.43	8.84
PR57	N/A	N/A	N/A	5.19	3.70	8.48
PR58	5.90	5.06	8.43	5.78	0.00	7.00
PR59	DROP	DROP	DROP	DROP	DROP	DROP

PVPI-F Group: Log Values of Microbiology Data
Baseline (S1) and 2 Months (S2), 6 Months (S3), and 1 Year (S4)

	S1			S2		
ID	logMS	logLB	logTVC	logMS	logLB	logTVC
PR03	6.38	5.90	8.69	5.13	5.14	8.30
PR04	5.62	5.06	8.53	6.04	3.78	8.15
PR07	5.03	3.70	7.95	5.14	4.62	8.15
PR08	6.04	6.08	8.68	4.43	2.05	8.28
PR09	6.11	4.62	8.61	5.85	4.53	8.49
PR10	4.86	3.46	7.90	5.56	0.00	8.46
PR16	2.43	2.78	7.00	3.30	2.79	8.71
PR19	5.09	5.05	8.26	6.38	5.53	8.58
PR20	6.18	4.36	8.58	5.85	1.61	8.49
PR22	6.32	4.86	8.38	6.23	4.88	8.28
PR23	5.27	4.91	8.30	5.29	4.51	7.95
PR25	5.90	5.08	8.08	5.60	5.00	7.00
PR26	4.46	2.96	8.43	5.23	3.05	8.26
PR29	0.00	0.00	8.40	0.00	0.00	7.95
PR30	0.00	3.16	8.49	0.00	2.59	7.95
PR31	4.63	5.43	8.75	6.34	5.78	8.77
PR33	4.28	3.48	8.11	4.70	4.18	8.15
PR34	5.03	4.81	7.78	6.15	5.47	8.79
PR36	4.08	0.00	8.04	4.40	2.23	8.15
PR39	5.60	4.85	7.00	5.53	4.80	8.11
PR41	6.74	3.85	8.93	6.63	4.18	9.10
PR43	6.30	3.06	8.62	4.04	0.00	8.18
PR47	4.94	2.96	7.85	5.18	4.28	8.30
PR49	6.85	5.38	8.20	5.19	4.94	8.88
PR50	5.45	1.04	8.92	5.30	1.85	9.03
PR51	5.70	0.00	8.04	6.48	1.79	8.90
PR52	4.62	0.00	7.60	3.95	0.00	7.30
PR56	5.47	3.60	8.20	4.91	2.30	8.04
PR60	6.57	4.30	8.88	5.70	2.85	8.61

ID	S3 log logMS	logLB	logTVC	S4 log logMS	logLB	logTVC
PR03	6.43	6.08	8.99	6.36	6.04	9.11
PR04	5.45	2.43	8.32	5.57	4.11	8.54
PR07	4.86	3.85	8.36	4.77	4.00	8.36
PR08	5.05	5.60	8.60	5.60	2.53	8.72
PR09	6.48	4.56	8.56	5.70	4.18	8.28
PR10	5.60	2.48	7.85	4.76	2.15	8.40
PR16	drop	drop	drop	drop	drop	Drop
PR19	5.13	4.32	7.60	5.48	4.54	7.90
PR20	5.70	2.75	8.11	4.53	2.90	7.30
PR22	4.76	2.05	8.53	5.33	1.85	8.20
PR23	5.31	3.60	7.95	4.69	3.85	7.90
PR25	6.36	6.61	8.34	6.52	6.32	8.80
PR26	6.15	4.32	8.52	6.11	4.85	8.98
PR29	0.00	1.04	7.78	0.00	0.00	8.15
PR30	0.00	2.76	8.38	0.00	3.00	8.54
PR31	6.53	3.78	8.70	6.32	5.37	9.05
PR33	3.95	2.94	7.85	3.85	3.26	7.78
PR34	6.28	5.48	8.45	4.57	5.60	8.62
PR36	3.60	0.00	8.00	4.38	0.00	8.15
PR39	2.93	2.71	7.95	5.60	4.77	7.48
PR41	6.30	4.26	8.72	6.57	4.82	8.46
PR43	4.04	0.00	8.32	4.64	0.00	8.20
PR47	5.42	4.40	8.15	5.30	4.68	7.78
PR49	4.91	5.95	8.52	4.93	6.36	8.23
PR50	5.70	0.00	8.81	5.40	1.61	8.81
PR51	6.63	0.00	8.89	5.13	0.00	8.95
PR52	4.15	0.00	8.43	4.15	0.00	8.00
PR56	5.48	5.53	8.53	5.49	3.85	8.59
PR60	drop	drop	drop	drop	drop	Drop

F Group: Difference in Log Values at 2 Months, 6 Months, and 1 Year

	S2-S1			S3-S1			S4-S1		
ID	logMS	logLB	logTVC	logMS	logLB	logTVC	logMS	logLB	logTVC
PR01	-1.08	-0.69	-0.22	-0.52	0.18	0.76	1.30	1.19	0.71
PR02	-0.52	-1.02	-0.19	-0.44	0.24	0.61	0.60	-0.59	0.61
PR05	0.20	-0.24	0.20	-0.07	0.00	0.00	-0.24	0.30	-0.41
PR06	0.58	0.58	0.27	0.72	-2.52	0.30	0.82	2.36	0.16
PR11	-0.31	1.79	0.00	0.38	0.00	0.60	-0.57	0.00	0.23
PR12	0.34	0.00	0.17	1.96	4.18	-0.16	1.41	4.45	0.43
PR13	-0.88	0.00	0.39	-0.28	0.00	-0.18	-0.02	1.85	-0.29
PR14	-0.47	-2.38	-0.35	0.06	-0.08	0.12	0.94	2.15	0.37
PR15	0.00	1.58	-0.17	0.67	3.56	-0.29	drop	drop	drop
PR17	1.31	0.00	0.14	0.30	1.79	-0.73	0.10	0.00	-0.63
PR 18	0.07	1.04	0.03	0.17	2.88	-0.30	0.22	5.34	0.32
PR 21	drop	drop	drop	drop	drop	drop	drop	drop	drop
PR 24	-0.51	-0.48	-0.23	-0.41	0.38	-0.56	0.02	-0.85	-0.30
PR 27	-0.21	0.00	-0.19	-0.34	2.88	-0.37	-0.40	0.00	0.42
PR 28	0.00	2.58	-0.32	0.00	2.67	-0.04	0.00	2.77	-0.32
PR 32	0.00	0.00	-0.44	0.00	0.00	0.04	0.00	0.00	-0.09
PR 35	-0.48	-0.79	0.28	-0.80	-2.43	0.69	-0.56	-2.03	-0.07
PR 37	-1.29	-1.66	-0.30	-0.11	0.07	0.00	-0.20	-0.04	0.40
PR38	-0.30	0.63	-1.31	-0.67	1.15	-0.98	0.43	1.33	0.41
PR40	0.34	0.49	-0.28	-1.36	0.22	-0.70	1.33	-1.48	-0.28
PR 42	-0.22	2.58	-0.79	0.21	3.79	-1.00	0.18	4.06	-0.96
PR 44	-1.49	-2.22	-0.41	n/a	n/a	n/a	0.09	-2.76	-0.01
PR45	-0.40	-1.00	-0.58	n/a	n/a	n/a	n/a	n/a	n/a
PR46	0.22	2.93	0.04	-0.28	2.52	0.44	-0.08	-2.32	0.38
PR48	-0.80	-1.40	-0.05	-0.02	-1.32	-0.18	0.40	-2.63	0.00
PR53	0.32	-3.00	0.06	0.00	-3.00	0.00	-0.08	-3.00	0.14
PR54	0.93	0.72	0.06	0.78	1.10	0.11	1.52	-0.43	0.46
PR55	-0.02	-0.17	0.30	0.22	-1.27	0.81	0.10	-0.62	1.24
PR57	0.53	-1.32	-0.37	n/a	n/a	n/a	-0.28	2.38	0.03
PR58	-0.99	0.55	-0.23	-0.10	0.73	0.05	-0.22	-4.32	-1.38
PR59	-0.40	0.00	-0.62	n/a	n/a	n/a	n/a	n/a	n/a

PVPI-F Group: Difference in Log Values at 2 Months, 6 Months, and 1 Year

	S2-S1			S3-S1			S4-S1		
ID	logMS	logLB	logTVC	logMS	logLB	logTVC	logMS	logLB	logTVC
PR03	-1.25	-0.77	-0.39	0.05	0.18	0.30	-0.02	0.14	0.42
PR04	0.42	-1.29	-0.39	-0.17	-2.63	-0.21	-0.05	-0.95	0.01
PR07	0.11	0.92	0.19	-0.17	0.15	0.41	-0.25	0.30	0.41
PR08	-1.61	-4.03	-0.40	-0.99	-0.48	-0.08	-0.44	-3.55	0.03
PR09	-0.27	-0.09	-0.12	0.36	-0.07	-0.06	-0.41	-0.45	-0.33
PR10	0.69	-3.46	0.56	0.74	-0.99	-0.06	-0.10	-1.31	0.49
PR16	0.86	0.01	1.71	drop	drop	drop	drop	drop	drop
PR19	1.29	0.48	0.32	0.04	-0.72	-0.65	0.39	-0.50	-0.35
PR20	-0.33	-2.75	-0.09	-0.48	-1.61	-0.47	-1.64	-1.46	-1.28
PR22	-0.09	0.01	-0.10	-1.57	-2.82	0.15	-1.00	-3.01	-0.18
PR23	0.02	-0.41	-0.35	0.04	-1.31	-0.35	-0.58	-1.07	-0.40
PR25	-0.31	-0.08	-1.08	0.46	1.54	0.26	0.62	1.25	0.72
PR26	0.77	0.09	-0.18	1.68	1.36	0.09	1.65	1.89	0.55
PR29	0.00	0.00	-0.44	0.00	1.04	-0.62	0.00	0.00	-0.25
PR30	0.00	-0.57	-0.54	0.00	-0.41	-0.11	0.00	-0.17	0.05
PR31	1.70	0.35	0.02	1.90	-1.65	-0.05	1.69	-0.06	0.30
PR33	0.42	0.70	0.03	-0.32	-0.54	-0.27	-0.43	-0.21	-0.34
PR34	1.11	0.66	1.01	1.25	0.67	0.67	-0.47	0.80	0.85
PR36	0.32	2.23	0.10	-0.48	0.00	-0.04	0.30	0.00	0.10
PR39	-0.07	-0.05	1.11	-2.67	-2.14	0.95	0.00	-0.08	0.48
PR41	-0.11	0.33	0.17	-0.44	0.41	-0.21	-0.17	0.97	-0.47
PR43	-2.26	-3.06	-0.45	-2.26	-3.06	-0.30	-1.66	-3.06	-0.42
PR47	0.23	1.31	0.46	0.48	1.43	0.30	0.35	1.72	-0.07
PR49	-1.66	-0.43	0.68	-1.93	0.58	0.31	-1.91	0.98	0.03
PR50	-0.15	0.81	0.11	0.25	-1.04	-0.11	-0.05	0.57	-0.11
PR51	0.78	1.79	0.86	0.93	0.00	0.85	-0.57	0.00	0.91
PR52	-0.67	0.00	-0.30	-0.48	0.00	0.83	-0.48	0.00	0.40
PR56	-0.56	-1.30	-0.16	0.01	1.93	0.33	0.02	0.24	0.39
PR60	-0.87	-1.46	-0.26	drop	drop	drop	drop	drop	drop

F Group: DMFS Data

ID	Primary Teeth #	ds	ss-ds	fs	ss-fs	ms	de-min	Perm Teeth #	DS	ss-DS	FS	ss-FS	MS	de-min
PR01	17	4	2	6	3	5	2	2	0	0	0	0	0	0
PR02	18	0	0	13	5	0	0	5	0	0	0	0	0	0
PR05	7	0	0	23	20	15	0	14	2	0	3	0	0	0
PR06	19	3	3	23	14	0	0	5	0	0	0	0	0	0
PR11	5	0	0	8	3	5	0	17	6	0	6	0	0	0
PR12	12	1	1	13	6	5	0	10	0	0	0	0	0	0
PR13	13	0	0	7	0	0	0	6	0	0	0	0	0	0
PR14	11	1	1	16	9	5	0	12	0	0	0	0	0	0
PR15	12	3	2	15	7	0	1	12	3	0	0	0	0	1
PR17	11	7	3	7	3	5	0	12	0	0	0	0	0	0
PR18	12	1	0	0	0	0	0	12	1	0	0	0	0	0
PR21	2	2	2	2	0	0	0	22	10	0	0	0	0	0
PR24	17	19	11	5	4	0	0	3	0	0	0	0	0	0
PR27	14	4	2	23	14	0	0	10	0	0	0	0	0	0
PR28	17	0	0	0	0	0	0	6	3	0	0	0	0	0
PR32	20	0	0	4	2	0	0	0	0	0	0	0	0	0
PR35	11	2	1	23	17	5	0	12	4	1	0	0	0	0
PR37	6	0	0	30	20	26	0	12	0	0	0	0	0	0
PR38	16	2	0	31	23	9	0	6	1	0	0	0	0	0
PR40	18	3	2	14	9	0	0	2	0	0	0	0	0	0
PR42	12	0	0	25	13	0	0	11	0	0	0	0	0	0
PR44	12	2	2	29	23	5	1	8	0	0	0	0	0	0
PR45	16	2	1	17	7	4	0	7	1	0	0	0	0	0
PR46	1	1	1	2	0	0	0	23	2	0	2	0	0	2
PR48	12	2	1	19	12	0	0	12	0	0	0	0	0	0
PR53	19	1	0	2	1	0	2	5	0	0	0	0	0	0
PR54	10	0	0	5	0	0	0	12	0	0	2	0	0	0
PR55	12	2	0	3	1	0	0	12	0	0	0	0	0	0
PR57	17	7	7	34	24	5	7	6	0	0	0	0	0	0
PR58	12	2	1	10	4	0	0	12	1	0	1	0	0	3
PR59	7	1	0	4	2	0	0	16	0	0	0	0	0	0

ID	Total Teeth #	ds/DS	ss-ds/DS	fs/FS	ss-fs/FS	ms/Ms	ss-dfs/DFS	dmfs/DMFS
PR01	19	4	2	6	3	5	5	15
PR02	23	0	0	13	5	0	5	13
PR05	21	2	0	26	20	15	20	43
PR06	24	3	3	23	14	0	17	26
PR11	22	6	0	14	3	5	3	25
PR12	22	1	1	13	6	5	7	19
PR13	19	0	0	7	0	0	0	7
PR14	23	1	1	16	9	5	10	22
PR15	24	6	2	15	7	0	9	21
PR17	23	7	3	7	3	5	6	19
PR18	24	2	0	0	0	0	0	2
PR21	24	12	2	2	0	0	2	14
PR24	20	19	11	5	4	0	15	24
PR27	24	4	2	23	14	0	16	27
PR28	23	3	0	0	0	0	0	3
PR32	20	0	0	4	2	0	2	4
PR35	23	6	2	23	17	5	19	34
PR37	18	0	0	30	20	26	20	56
PR38	22	3	0	31	23	9	23	43
PR40	20	3	2	14	9	0	11	17
PR42	23	0	0	25	13	0	13	25
PR44	20	2	2	29	23	5	25	36
PR45	23	3	1	17	7	4	8	24
PR46	24	3	1	4	0	0	1	7
PR48	24	2	1	19	12	0	13	21
PR53	24	1	0	2	1	0	1	3
PR54	22	0	0	7	0	0	0	7
PR55	24	2	0	3	1	0	1	5
PR57	23	7	7	34	24	5	31	46
PR58	24	3	1	11	4	0	5	14
PR59	23	1	0	4	2	0	2	5

PVPI-F Group: DMFS Data

ID	Primary Teeth #	ds	ss-ds	fs	ss-fs	ms	de-min	Perm Teeth #	DS	ss-DS	FS	ss-FS	MS	de-min
PR03	14	0	0	24	15	10	2	11	1	0	0	0	0	0
PR04	18	0	0	17	13	0	0	4	0	0	0	0	0	0
PR07	12	0	0	10	1	0	0	12	2	2	0	0	0	0
PR08	2	0	0	5	2	0	0	19	0	0	0	0	0	9
PR09	11	3	1	2	1	5	0	10	2	0	0	0	0	0
PR10	12	3	2	10	7	0	0	12	0	0	0	0	0	0
PR16	13	3	3	6	2	0	0	10	2	0	0	0	0	0
PR19	5	2	1	11	9	14	0	15	4	1	6	0	0	0
PR20	12	0	0	27	17	0	0	6	2	0	0	0	0	0
PR22	8	2	1	8	4	0	0	15	6	0	0	0	0	0
PR23	12	0	0	30	19	0	0	10	0	0	1	0	0	0
PR25	8	2	2	14	9	36	0	8	2	0	2	0	0	0
PR26	11	0	0	33	22	5	0	13	2	0	0	0	0	0
PR29	20	2	0	0	0	0	0	0	0	0	0	0	0	0
PR30	12	0	0	3	1	5	0	10	4	0	0	0	0	0
PR31	11	10	5	0	0	5	0	12	0	0	0	0	0	8
PR33	10	0	0	6	3	5	0	12	0	0	0	0	0	0
PR34	13	4	2	9	5	15	0	10	0	0	0	0	0	0
PR36	12	2	0	11	4	0	0	12	0	0	0	0	0	0
PR39	10	2	1	8	4	0	0	12	1	0	2	0	0	0
PR41	12	1	1	30	21	0	0	12	0	0	0	0	0	3
PR43	0	0	0	0	0	0	0	23	1	0	6	0	0	0
PR47	14	0	0	4	2	0	0	10	0	0	0	0	0	0
PR49	14	15	8	16	7	0	0	10	5	0	0	0	0	0
PR50	21	9	5	13	7	0	3	2	0	0	0	0	0	0
PR51	14	10	10	0	0	0	0	6	0	0	0	0	0	0
PR52	10	2	2	0	0	0	0	12	0	0	0	0	0	0
PR56	16	4	2	10	3	0	0	6	0	0	0	0	0	0
PR60	14	12	8	18	8	5	5	9	0	0	1	0	0	0

	Total Teeth #	ds/DS	ss-ds/DS	fs/FS	ss-fs/FS	ms/Ms	ss-dfs/DFS	dmfs/DMFS
PR03	25	1	0	24	15	10	15	35
PR04	22	0	0	17	13	0	13	17
PR07	24	2	2	10	1	0	3	12
PR08	21	0	0	5	2	0	2	5
PR09	21	5	1	2	1	5	2	12
PR10	24	3	2	10	7	0	9	13
PR16	23	5	3	6	2	0	5	11
PR19	20	6	2	17	9	14	11	37
PR20	18	2	0	27	17	0	17	29
PR22	23	8	1	8	4	0	5	16
PR23	22	0	0	31	19	0	19	31
PR25	16	4	2	16	9	36	11	56
PR26	24	2	0	33	22	5	22	40
PR29	20	2	0	0	0	0	0	2
PR30	22	4	0	3	1	5	1	12
PR31	23	10	5	0	0	5	5	15
PR33	22	0	0	6	3	5	3	11
PR34	23	4	2	9	5	15	7	28
PR36	24	2	0	11	4	0	4	13
PR39	22	3	1	10	4	0	5	13
PR41	24	1	1	30	21	0	22	31
PR43	23	1	0	6	0	0	0	7
PR47	24	0	0	4	2	0	2	4
PR49	24	20	8	16	7	0	15	36
PR50	23	9	5	13	7	0	12	22
PR51	20	10	10	0	0	0	10	10
PR52	22	2	2	0	0	0	2	2
PR56	22	4	2	10	3	0	5	14
PR60	23	12	8	19	8	5	16	36

F Group: Age, Gender and Ethnicity

ID	age	gender	ethnicity
PR01	6	F	Arabic
PR02	6	F	Arabic
PR05	9	M	CenAme
PR06	6	M	Mid Eas
PR11	9	F	Chinese
PR12	8	M	AA
PR13	7	M	CenAme
PR14	8	F	Mex
PR15	8	F	Mex
PR17	8	F	Mid Eas
PR18	7	F	Mid Eas
PR21	9	M	AA
PR24	6	M	Filipino
PR27	7	M	SAmer
PR28	7	M	Mex
PR32	6	M	Mex
PR35	9	M	Mex
PR37	7	F	CenAme
PR38	6	M	Mex
PR40	6	M	Mex
PR42	7	M	AA
PR44	6	F	Korean
PR45	6	M	Euroasian
PR46	8	F	AA
PR48	8	M	Mex
PR53	6	F	Mex
PR54	9	F	Mid Eas
PR55	8	F	Mid Eas
PR57	7	M	CenAme
PR58	8	F	Mex
PR59	8	F	Arabic

PVPI-F Group: Age, Gender and Ethnicity

ID	age	gender	ethnicity
PR03	8	F	Arabic
PR04	6	M	Mex
PR07	8	M	Mex
PR08	9	M	Mex
PR09	8	F	CenAme
PR10	8	M	Cauc
PR16	7	F	Mex
PR19	7	F	Mex
PR20	6	F	Mex
PR22	7	M	AA
PR23	7	M	Filipino
PR25	6	F	Mex
PR26	9	M	
PR29	6	M	Mex
PR30	8	M	Mex
PR31	8	M	CenAme
PR33	8	M	Mex
PR34	6	M	Filipino
PR36	8	M	Filipino
PR39	8	F	SAmer
PR41	8	M	CenAme
PR43	9	F	Mex
PR47	6	F	Cau/Ch/fili/
PR49	7	M	Mex
PR50	6	M	CenAme
PR51	7	M	CenAme
PR52	9	M	Mex
PR56	8	M	Arabic
PR60	6	M	Arabic

F Group: Questionnaire Data

ID	Brushing frequency/day	Toothpaste used	Brand	Snacking frequency/day	Flossing Frequency/week	Seen dentist	Know result?
PR01	2	Y	Crest	1	1	Y	Y
PR02	2	Y	Crest	2	Daily	Y	Y
PR05	2	Y	Colgate	1	0	Y	Y
PR06	2	Y	Crest	2	1	Y	Y
PR11	2	Y	Crest	2	Daily	Y	Y
PR12	2	Y	Crest	1	2	Y	Y
PR13	2	Y	Colgate	2	0	Y	Y
PR14	2	Y	Colgate	3	3 to 6 times	Y	Y
PR15	2	Y	Crest	3	0	Y	Y
PR17	2	Y	Colgate	2	0	Y	Y
PR18	1	Y	Colgate	3	0	Y	Y
PR21	1	Y	any with FL	3	1	N	Y
PR24	2	Y	Colgate,Crest	3	1	Y	Y
PR27	2	Y	Closeup	1	0	Y	Y
PR28	2	Y	Colgate	3	1	Y	Y
PR32	2	Y	Colgate Total	1	Daily	Y	Y
PR35	1	Y		1	3 to 6 times	Y	Y
PR37	2	Y	Colgate	3	1	Y	Y
PR38	1	Y	Crest	1	0	Y	Y
PR40	2	Y	Crest	3	3 to 6 times	Y	Y
PR42	2	Y	Colgate	2		Y	Y
PR44	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PR45	Not every Day	Y		2	3 to 6 times	Y	Y
PR46	2	Y	Colgate	2	1	Y	Y
PR48	2	Y	Oral B	1	N/A	N/A	N/A
PR53	1	Y	Colgate	1	2	Y	Y
PR54	2	Y	Colgate	1	7	Y	Y
PR55	2	Y	Colgate	1	7	Y	Y

PR57	2	Y	Colgate	1	1	Y	Y
PR58	2	Y	Colgate	2	1	Y	Y
PR59	1	don't know	Tom's	1	0	Y	Y

PVPI-F Group: Questionnaire Data

ID	Brushing frequency/day	FL Toothpaste used	Brand	Snacking frequency/day	Flossing frequency/week	Seen Dentist	Know Result?
PR03	2	Y	Crest	1	1	Y	Y
PR04	1	Y	Colgate	1	don't Know	Y	Y
PR07	2	Y	Colgate	2	1	Y	Y
PR08	1	Y	Crest	2	1	Y	Y
PR09	2	Y	OralB	1	2	Y	Y
PR10	2	Y	N/A	2	Daily	Y	Y
PR16	2	Y	Colgate	2	2	Y	Y
PR19	2	Y	N/A	2	2	Y	Y
PR20	2	Y	N/A	2	2	Y	Y
PR22	2	Y	Colgate	3	not at all	Y	Y
PR23	2	Y	Colgate	2	1	Y	Y
PR25	2	Y	Colgate	1	Daily	Y	Y
PR26	2	Y	Colgate	2	N/A	N/A	N/A
PR29	2	Y	Colgate	3	1	Y	Y
PR30	2	Y	Colgate	1	0	Y	Y
PR31	2	N	Colgate	3	N/A	Y	Y
PR33	2	Y	Colgate Total	1	Daily	Y	Y
PR34	2	Y	Crest	1	don't Know	Y	Y
PR36	2	Y	kids Col/k crest	3	2	Y	Y
PR39	2	Y	Crest	3	3 to 6 times	Y	Y
PR41	Not every day	Y	Colgate	2	not at all	Y	Y
PR43	1	Y	Crest	3	Daily	Y	Y
PR47	2	Y	Crest	2	7+	Y	Y
PR49	2	Don't Know	N/A	3	2	Y	Y
PR50	2	N	Colgate	2	not at all	Y	Y
PR51	2	N	Colgate	2	not at all	Y	Y
PR52	1	Y	Colgate	1	1	Y	Y
PR56	1	Y	Colgate	1	7	Y	Y

PR60	Not Answered	Don't Know	Tom's	1	not at all	Y	Y

11. Appendix B: CHR and Forms

UCSF

Please date form: 07/19/05

COMMITTEE ON HUMAN RESEARCH

FULL COMMITTEE REVIEW APPLICATION

General Instructions | View Complete Set of Linked Instructions

PART 1: ADMINISTRATIVE REQUIREMENTS

- Eligibility requirements for Principal Investigator, Co-Principal Investigator and Contact Person
- Training requirements

A. Principal Investigator:		
Name and degree John D. B. Featherstone, PhD., M.Sc.	University Title Professor	Department Prev & Rest Dental Sciences
Campus Mailing Address (Box No.) Box 0758	Phone Number 415/ 476-0456	E-mail Address jdbf@ ucsf.edu
Co-Principal Investigator:		
Name and degree Ling Zhan, DDS, PhD	University Title Postdoctoral Researcher	Department Prev & Rest Dental Sciences
Campus Mailing Address (Box No.) Box 0758	Phone Number 415/476-0921	E-mail Address zhanl@dentistry.ucsf.edu
Additional Contact Person (if any):		
Name	University Title	Department
Campus Mailing Address (Box No.)	Phone Number	E-mail Address

Send correspondence to (check *one*):	[ ]PI only [X]PI and Co-PI [ ]PI and Additional Contact Person

Study Title:	Application Type:
Effectiveness of Specific Antimicrobial Treatment Against Bacteria that Cause Dental Decay in Children in Pacific Rim Countries	[]New Full Committee Application []Response to "Contingent" or "Return" letter [X]Modification [ ]Renewal Current CHR #: __ Expiration date: __

Sites (Check all that apply):

[X]UCSF [ ]SFGH [ ]VAMC [ ]Fresno
[ ]Cancer Center [ ]UC Berkeley
[ ]GCRC (Moffitt/Mt. Zion) [ ]GCRC (SFGH) [ ]PCRC
[X]Foreign Country
[ ]Other(s):

B. Funding: If this study is eligible for "Just in Time" NIH review, do not submit your application to the CHR until you have received notification from the federal granting agency that your study appears to be in a fundable range. Check all that apply:

Type of funding	Source of funding	Funds will be awarded to/through:
[X]Contract/Grant [ ]Subcontract [ ]Drug/device donation [ ]Student project [ ]Other: __ Have funds been awarded? [X]Yes[ ]Pending	[ ]Federal Government [ ]Other Gov. (e.g., State, local) [ ]Industry* [ ]Other Private [X]Campus/UC-Wide program [ ]Departmental	Dept./ORU: *InstitutionFederal Wide Assurance* [X]UCSF ..... 0 [ ]Blood Centers of the Pacific.... 0 [ ]Gallo Institute ..... 0 [ ]Gladstone Institute ..... 0 [ ]Goldman Institute on Aging..... 0 [ ]NCIRE ..... 0 [ ]S.F. Dept. of Public Health..... 0

[ ]No Award No.: 04TPRRP 02-0012	Funds [ ]Other: Sponsor Name: UC Pacific Rim Research Program and IADR/GSK innovative research award (see below)	[ ]VA Research Office..... 0
*UCSF (or affiliate) financial contact person for IRB review recharge:	Normita Santore, AC Phone: 4-0495 AC Fax: 6-0858 AC E-mail: santore@itsa.ucsf.edu	
Grant Title and PI (if different from above):		
Secondary sponsors: If there are multiple sources of funding for this study, please describe the additional funding:		
IADR/GlaxoSmithKline Innovation in Oral Care Awards Title: A NOVEL ANTIBACTERIAL APPROACH TO REDUCE CARIES IN CHILDREN		

C. Key Personnel: All key personnel including the PI and Co-PI must be listed below along with a brief statement of their qualifications. *If the SF VAMC is a study site*, please identify the principal VAMC investigator, unless already listed as PI or CoPI above. For questions regarding the VAMC application process, please contact the VA Clinical Research Office at 221-4810 ext.4655.	
Investigator (and institution):	Qualifications:
John Featherstone (PI), UCSF	Dr. John D.B. Featherstone is professor and Chair of the Department of Preventive and Restorative Dental Sciences at UCSF. Dr. Featherstone is internationally recognized for studies relating to de- and remineralization of the teeth, and clarification of the mechanisms of action of fluoride in preventing or reversing dental caries. He has been principal

Ling Zhan(co-PI), UCSF	investigator or co-investigator on numerous NIH/NIDR funded studies. Dr. Ling Zhan is now a postdoctoral researcher in the Department of Preventive and Restorative Dental Sciences at the UCSF. She came from West China College of Stomatology as a visiting professor 4 years ago. She is a trained dentist with over 10 year clinical practice experience. Her research career has been focused on microbiology aspects of caries and caries prevention. She has led one pilot project funded by NIH/NIDCR on cariogenic bacteria in children, and
Pamela Den Besten (co-investigator), UCSF	she has participated in several clinical studies related to caries risk assessment and caries prevention by antibacterial agents in children and adults funded by NIH/NIDCR. She is Professor and Chair of the Division of Pediatric Dentistry at UCSF and has 20 years of clinical and research experience. Dr. Den Besten was the
Purvi Zavery (co-investigator, UCSF)	principal investigator on two key studies using PVP-I to reduce MS and LB bacteria in children with early childhood caries (ECC). She will be co-investigator responsible for the overall clinical aspects of the study at UCSF. Dr. Zavery is a resident in the pediatric dentistry specialty program at UCSF. She will work closely with Dr. Zhan and will be responsible for recruiting, scheduling subjects, clinical treatment of the subjects, applying the test agents, and will actively participate in data analyses.
XueDong Zhou(Co-investigator), West China College of Stomatology (WCCS)	Dr. Zhou is the dean of the college and the chair of the Operative Dentistry Department at WCCS. She is internationally recognized for her research in caries etiology and prevention and is now the president of Chinese Association of Caries Research. She has been principal investigator and co-investigator on

	numerous caries research projects funded by the Chinese government and international foundations. She is co-investigator of the study and will be responsible for leading the study at WCCS.

D. Drugs, Devices and Biologics:		
List any investigational drugs, biologics and IND Numbers:	name	IND #
List any investigational devices and IDE Numbers:	name	IDE#
	[ ] Non-Significant Risk Determination Requested Attach NSR Supplement	
Who holds the IND/IDE?	[ ]Sponsor [ ]Investigator	
List any approved drugs, biologics and/or devices being *studied*:	10% povidone iodine solution 2% sodium fluoride foam	
Are investigational drugs, devices, or biologics prepared or manufactured in UCSF research labs?	[ ]Yes [X]No If "Yes," identify the lab:	

E. Other Approvals/Regulated Materials: Does this study require approval or authorization from any of the following regulatory committees, or involve the use of the regulated materials listed below? Follow the hyperlinks for more information. If "Yes," complete the applicable section(s) below.			[ ]Yes [X]No
[x]	Biological Safety Committee		BUA #: 2308-BU-01-INC (expiration 4/1/06)
	[ ]	Gene Transfer/Therapy	
[ ]	Institutional Animal Care and Use Committee		IACUC #:
	[ ]	Xenotransplantation Clinical Trial	
[ ]	Controlled Substances		
[ ]	Human Stem Cells		Attach Stem Cell

			Supplement
	[ ]	Embryonic Stem Cell Clinical Trial	
[ ]	Radiation Safety Committee		RUA #:

F. Scientific Merit Review: This study has received or will receive scientific merit review from (check all that apply):
[ ]NIH [ ]Cancer Center* [ ]GCRC or PCRC [ ]SFVAMC [ ]Dept. Review [
*Required prior to final CHR approval for oncology studies.

G. Statement of Financial Interest: Do you or the other investigators have a financial interest in the outcome of this study? If "Yes," please describe below and describe briefly in Purpose and Background section of the consent form.	[ ]Yes [X]No

H. Principal Investigator's Certification:

- I certify that the information provided in this application is complete and correct.
- I accept ultimate responsibility for the conduct of this study, the ethical performance of the project, and the protection of the rights and welfare of the human subjects who are directly or indirectly involved in this project.
- I will comply with all policies and guidelines of UCSF and affiliated institutions where this study will be conducted, as well as with all applicable federal, state and local laws regarding the protection of human subjects in research.
- I will ensure that personnel performing this study are qualified, appropriately trained and will adhere to the provisions of the CHR-approved protocol.
- I will not modify this CHR-certified protocol or any attached materials without first obtaining CHR approval for an amendment to the previously approved protocol.
- I assure that the protected health information requested, if any, is the minimum necessary to meet the research objectives.
- I assure that the protected health information I obtain, if any, as part of this

research will not be reused or disclosed to any parties other than those described in the CHR-approved protocol, except as required by law.

Principal Investigator's Signature Date

PART 2: STUDY DESIGN

Complete items A-E using clear, concise, non-technical, lay language (i.e., the type of language used in a newspaper article for the general public) wherever possible. Define all acronyms. Use caution when cutting and pasting from another application or protocol to ensure that information is complete, supplemented where necessary, is pasted in a logical order, and is relevant to the specific section.

Space limits are recommendations and should be adjusted as needed, but the total length for sections A-E should not exceed 5 pages.

For modifications and renewals, please highlight in *italics* all changes from previously approved version.

A. Synopsis (Briefly summarize the study.) Sp

Aim: The aim of the study is to conduct a one year randomized clinical study to determine the efficacy of four weekly applications of a povidone iodine/fluoride (PVP-IF) foam at baseline and 6 months on the reduction of oral cariogenic bacteria levels (mutans streptococci (MS) and Lactobacillus (LB) species), the genetic diversity of MS, and caries increment in children with active caries. The hypothesis to be tested is that bi-annual four weekly application of PVP-IF will suppress MS and LB colonization, decrease the genetic diversity of MS, enhance remineralization and reduce future caries formation. Methods: 60 healthy 6-9 year olds with 1-5 frank caries lesions will be randomized to 30 per each of the intervention and control groups, namely a) control: F-only foam group or b) intervention: the PVP-IF foam group. Treatments will be performed at baseline and then once a week for 3 consecutive weeks. The four weekly treatments will be repeated 6 months after enrollment. Stimulated saliva

samples will be taken at the initial visit before treatment, then 1 month, 5 months, and 1 year after the first four weekly treatments are complete and assayed for MS, LB, and MS genetic diversity. The DMFT and DMFS (decayed missing and filled teeth and surfaces) will be recorded using NIDCR modified WHO criteria at the initial treatment and at the 1 year final visit by one examiner. Quantitative light florescent (QLF) images on occlusal and buccal surfaces of molars for early caries lesion detection will also be used to augment the examination. The instrument includes a repositioning software to enable a 95% positioning match for each re-assessment. Ecological shifts in selected flora will be followed by checkerboard analyses at baseline, 1 month and 1 year. Significance: Potentially a PVP-IF combined foam could be applied to the teeth of children at appropriate intervals throughout a year, reducing both the MS and LB bacterial challenge, enhancing remineralization, and markedly reducing or even eliminating new caries formation. If the hypothesis is proven, this novel PVP-IF foam will be a convenient antibacterial treatment as a strategy in caries prevention for children in U.S.A. and beyond, especially for children with high caries risk from socioeconomically disadvantaged families.

B. Purpose (Specify the hypotheses, aims and/or objectives.)

The aim of the study is to conduct a one year randomized clinical study to determine the efficacy of four weekly applications of a novel povidone iodine/fluoride (PVP-IF) foam at baseline and 6 months on the reduction of oral cariogenic bacteria levels (namely mutans streptococci (MS) and *Lactobacillus* (LB) species), the genetic diversity of mutans streptococci, and caries increment in children with active caries. The hypothesis to be tested is that bi-annual four weekly application of PVP-IF will suppress MS and LB colonization, decrease the genetic diversity of MS, enhance remineralization and reduce future caries formation.

C. Background (Summarize previous studies. Explain rationale for the proposed investigation.)

Prevalence of dental decay worldwide and in the United States

Dental caries continues to be a major oral health concern in children in the USA and world wide. The third National Health and Nutrition Examination

Survey-Phase I showed 50% of 5-8 year old children in the US had experienced caries in the primary dentition (baby teeth) and 67% of 12-17 year olds had experienced caries in the permanent dentition [1]. Remarkably, 11% of children aged 6-11 years had 75% of the caries[2].

Caries prevention measures and antibacterial therapy

Dental caries is an infectious disease caused by acid producing bacteria, primarily MS, and LB in adults and children[3]. Its initiation and progression depend on a balance between demineralization of the enamel, by acids produced by cariogenic bacteria, and remineralization by calcium and phosphate from saliva enhanced by fluoride. Fluoride from drinking water and dental products has played a considerable role in the reduction of caries prevalence and its severity during the past few decades[3]. However fluoride therapy alone can not overcome the severe bacterial challenge in high caries risk individuals.

In addition to cariogenic bacteria quantity, recent studies by us and others found that high caries active children and adults displayed high genetic diversity (numerous different strains) in MS infection[4-6]. Studies have shown that "restoring" carious lesions has a minimal effect on the bacterial loading in the remainder of the mouth. High-risk individuals therefore need antibacterial treatment to reduce their "caries challenge" in both quantity and virulence. Although the concept of using antibacterial therapy is logical, it is not much used in the prevention of tooth decay[3]. To date, there are no specific effective antibiotics or vaccines available against cariogenic bacteria.

Chlorhexidine has been the most often used antibacterial for caries prevention, but it has yielded limited success. Chlorhexidine reduces the levels of MS in the mouth if used daily for two weeks, but its efficacy on lactobacilli is limited[3]. The unpleasant taste and staining reduce patient compliance. Therefore, an antibacterial with increased efficacy against cariogenic bacteria with improved patient acceptance is needed.

PVP-I (10% povidone-iodine, with 1% active iodine) has been approved for application to the skin and mucous membranes of children in general clinical practice, as a pre-surgical antiseptic, and is considered safe for intraoral use. It appears to be a promising antibacterial for cariogenic bacterial infection. Topical use of iodine showed prolonged suppressive effects on oral populations of MS[7]. Very importantly, a recent study by Lopez et al showed

that bi-monthly topical application of a 10% povidone iodine solution to the dentition of babies at high risk for early childhood caries (ECC) prevented the development of white spot lesions[8].

PRELIMINARY RESULTS: (pilot studies, unpublished)

a) A pilot study(CHR approval # H8693-17428-01) at UCSF with 20 children showed that a single application of PVP-I solution following treatment for early childhood caries in children under 5 years of age, under general anesthesia after restoration of all active caries, reduced MS and LB levels significantly for up to 3 months[9], but the one time application did not reduce caries incidence over 12 months. B) We have just completed a pilot study (CHR approval # H8693-22998-01A) using PVP-I foam on children (aged 6 to 9 years) with active caries. The fruit flavored PVP-I foam gave reduced MS and LB levels at 1 week compared to baseline, but the effects were diminished by 1 month. Further, the PVP-I foam formulation was well accepted by the participating children. These results suggest that four PVP-I foam treatments repeated at weekly intervals will likely be a good treatment regimen to substantially reduce or eliminate MS and LB. c) A study (CHR approval # H8693-22805-01) in high caries and caries free children showed that MS infection diversity (number of strains in one individual) is positively correlated with ds (decayed surfaces) scores of subjects. These results indicate that genetic diversity may contribute to MS virulence[5, 6]. D) We have just completed a five year NIH-funded study on "Caries management by risk assessment" in adults(CHR approval #H9136-13891-03A). Results showed the cariogenic bacterial challenge remained high despite the completion of conventional dental treatment[10], unless aggressive antibacterial therapy was used in conjunction with restorative work in high caries individuals.

However, PVP-I has not frequently been used in full mouth dental rehabilitation and its related efficacy in decreasing the risk for recurrent decay by multiple applications is unknown. Furthermore the beneficial effects of a high concentration fluoride (5,000 ppm F) foam or gel are well known in enhancing remineralization of caries lesions. There is no chemical reason why we can not combine PVP-I and F in one treatment regimen. This novel approach will be used in the present study.

D. Design

General Study Design and inclusion criteria:

The study will be carried out at two sites, namely (1) UCSF, and (2) West China College of Stomatology (WCCS). The study at UCSF will be carried out at the Predoctoral Pediatric Dental Clinic (PDPDC) and the Postgraduate Pediatric Dental Clinic (PGPDC). The study at WCCS will be carried out at the Operative Dentistry clinic. A separate human subject approval will be obtained in China for the study conducted at WCCS, and it will use the identical protocol. A total of 60 healthy 6-9 year olds with 1-5 frank caries lesions (who assent and their parents give consent) will be enrolled in the study at each site, randomized to 30 per each of the test and control groups. Eligible children who reside within a 25 mile radius will be randomly assigned to one of the two study groups, a) control: F-only foam group or b) intervention: the PVP-IF foam group. The corresponding treatment will be performed at baseline and then once a week for 3 consecutive weeks. The four weekly treatments will be repeated 6 months after enrollment. Stimulated saliva samples will be taken at the initial visit before treatment, then 1 month, 5 months, and 1 year after the first four weekly treatments are complete. The investigator will coordinate with the PDPDC and PGPDC department to have all cavities of the enrolled subjects treated within 6 month after enrollment as they are indicated in the conventional treatment plan. The DMFT and DMFS will be recorded using NIDCR modified WHO criteria at the initial treatment and at the 1 year final visit by one examiner and with the aid of radiographs. The quantitative light fluorescent (QLF) images of occlusive and buccal surfaces of molars will be taken at the oral examination visits to evaluate early non-cavitated lesions and to augment the visual exams.

Dr. Ling Zhan is from WCCS and is currently a postdoctoral fellow at UCSF. She will act as the liaison between the two sites. She is competent in all aspects of the study, including the microbiology. She will work with Dr. Den Besten (see below) at UCSF in subject recruitment and clinical treatment procedures, and will work with personnel in Dr. Hoover's lab at UCSF for microbiology plating and enumeration. Dr. Ling Zhan will be responsible for supervising these procedures when she travels to WCCS in the early part of the study year. We have the unique opportunity to capitalize on the knowledge and experience of Dr. Zhan to commence this exciting collaboration between the two universities. She will return to UCSF during the year of the study to coordinate between the two sites.

Sample size: Based on our study on ECC children (see above) we predict that 65% of subjects in the control group will have new caries within a year. For a predicted 30% reduction in new caries in the PVP-IF group the sample size needed to detect that difference at alpha (two

sided)= 0.05, power 80%, is estimated as 26 (Chi-square test) per group. We will use 30 subjects in each group to allow for about 15% attrition.

Human Subjects Approval. UCSF Committee on Human Research (CHR) approval will be obtained prior to recruitment of any children at UCSF. The CHR has previously approved the use of PVP-IF foam and salivary assays for our pilot studies. Payments to the subjects (children) and their parent will be as per the payment schedule table below. At UCSF, each child will receive a $10 gift card and the parent/guardian will receive $10 cash for each treatment and saliva visit. Both of the child and his/her parent/guardian will get $4 per visit cash bonus at the final visit that will be $40 cash for each of them if they complete all ten study visits. That is a total of $280 reimbursement for each subject and their parent/guardian if they complete the study.

Parents or legal guardians of eligible subjects will receive an explanation of the study procedures. If they agree to allow their children to participate, they will be asked to sign an informed consent document approved by the UCSF committee on human research (CHR). The CHR approval from WCCS will be obtained before any children are enrolled in China.

Exclusion criteria are children: a).with serious chronic systemic or periodontal diseases; b).with medicines taken within the past 3 months that might affect oral flora; c).with a dry mouth or difficulty spitting

Blinding. Placebo foam (fluoride only, control group) with similar color to the PVP-IF will be used and containers for collecting saliva will be uniquely coded. Only the statistician will know subject group assignments.

PVP-IF and F foams: A pharmaceutical company (Omnii Oral Pharmaceuticals, West Palm Beach, FL) has agreed to collaborate with us and to provide the prototype PVP-IF foam (1% active I and 5000 ppm F) and F-foam (5000 ppm F) for the study free of charge. The company has already supplied the product for our pilot studies described above.

Clinical Procedures and Saliva Sample Collection. At UCSF, assenting eligible subjects will be scheduled for the initial visit. Before any treatment, 2 ml of paraffin-stimulated saliva will be collected for microbiological assessment, including the checkerboard assessment. An oral examination will be conducted by Dr. Zhan, who is trained in the use of WHO criteria, and the DMFS and DMFT will be recorded.(Fifteen subjects from the study will be re-examined to assess the reliability). The subject will then receive either F-foam or PVP-IF foam treatment in pre-made trays for 4 minutes, and excess foam will be removed by suction. The patient will be instructed not to rinse for 30 minutes after treatment. The foam treatment will be repeated three times, once weekly for the next 3 weeks, and four times at weekly intervals 6 months after

enrollment. Three additional saliva samples will be collected at 1 month, 5 months and 12 months after the first four weekly treatments, for bacterial assays.
A pilot tested questionnaire will be handed out at each foam treatment to ask the child to evaluate their acceptance of the PVP-IF treatment. Potential side-effects and complaints of the treatment will be recorded by the investigators at each visit. After subjects are recruited into the study, a treatment plan will be written to include all detected cavities to be treated in six months at UCSF after the initial visit. A second caries examination for each subject will be done by the same examiner at the one year follow-up visit
Microbiology. Saliva samples will be sonicated for 20 seconds, and an aliquot will be plated within 24 hours of collection on MSSB agar for MS, on Rogosa agar for LB and on rabbit blood agar for TVC (Total Viable Counts). Plates will be incubated in 85% N_2, 10% H_2, & 5% CO_2 for 48 hours before enumeration of bacterial colonies. Five typical MS colonies will be isolated from MSSB agar based on the colony morphology. Fermentation tests will be used for MS identification. The strains confirmed as MS will be stored in glycerol TSB broth at -80°C.
Checkerboard assay. 1 ml of the saliva sample will be added to a buffer containing 0.25M NaOH, 5 mM Tris, 0.5 mM EDTA and stored at -20 deg C prior to shipping frozen to the Sissons lab for checkerboard analysis according to Wall-Manning, Sissons et al., 2002[11]. A range of oral bacterial species will be assessed and the ecological shifts determined over time.
AP-PCR assay will be used to determine the genetic diversity (# of species & strains within a single subject). In brief, stored MS isolates will be cultured in TPY broth. The bacterial DNA will be extracted by QIAamp DNA Mini Kit (Qiagen Sciences, Maryland, USA). Five μL of the extracted DNA will be place in a standard AP-PCR reaction buffer with 1 single primer (primer OPA-5: 5'-AGGGGTCTTG-3' or primer OPA013: 5'- CAGCACCCAC-3', Invetrogen, U.S.A.). Following 35 PCR amplification cycles the products will be analyzed by agarose gel electrophoresis. The DNA fragment banding patterns observed allow us to differentiate between MS species & strains.
1. (Check all that apply):

[ ]Phase I [ ]Phase II [ ]Phase III [ ]Phase IV [X]Randomized [X]Blinded

[X]Multicenter: If so, is UCSF the coordinating center? [X]Yes [ ]No

[ ]Open Label Extension: If so, specify CHR Approval Number for original study: __

[ ]Behavioral

2. Additional description of *general* study design. Attach flow diagram if appropriate.Space limit: h

E. Data Analysis (How and by whom will data be analyzed?)

The DMFS increment and percentage of the subjects with new decay will be calculated in both groups. The difference in DMFS increment between the two groups will be analyzed by the 2 sample Student t test. The percentage of the subjects with new decay will be analyzed by the Chi-square test. The QLF parameters of lesion area and fluorescence will be similarly analyzed. Counts of MS, LB and TVC (CFU/ml saliva) will be determined for each time point. Regression analyses (linear or logistic) will be used to test differences while adjusting for covariates such as age and gender. For each subject the reduction in logMS and logLB will be calculated by comparing the logarithms of the pre-treatment bacterial counts with logarithm of the bacterial counts at each time point. The two mean log reductions will be compared to determine statistical differences at the initial visit, 6 month and 12 month follow-up to

calculate the long term impact of the repeated PVP-IF treatment on cariogenic bacteria recolonization. For each subject, the changes of MS amplitypes at 1month and 1 year after the 1st four weekly treatments will be calculated against baseline. The mean and standard deviation from the change of amplitypes will calculated for both groups. The two means will be compared to determine statistical differences at the initial visit, 1 month and 12 month follow-up to calculate the long term impact of the repeated PVP-IF treatment on MS infection diversity.

Dr. Stuart Gansky will be responsible for overseeing the study design, data management, generating data analysis plan and performing multifactor regression analysis. Dr. Ling Zhan will be responsible for data entry and simple data analysis under Dr. Gansky's guidance.

PART 3: PROCEDURES

A. Check all that apply.

[ ] Human Biological Specimen Banking... Attach Banking Supplement

[ ] Genetic Testing [ ] HIV Testing

B. Please list, in sequence, all study procedures, tests, and treatments required for the study. Indicate which would be done even if a subject does not enroll in the study. Include a detailed explanation of any experimental procedures. Attach table if available.

Refer to flow chart in section 2 above for a summary of all procedures in sequence.

C. List the clinics and/or other specific locations where study procedures will be performed. Indicate how much time will be required of the subjects, per visit and in total for the study.

The study at UCSF will be carried out at the Predoctoral Pediatric Dental Clinic (PDPDC) and Postgraduate Pediatric Dental Clinic(PGPDC) at 707 Parnassus Ave. The study at WCCS will be carried out at the Operative Dentistry Clinic. A copy of the Chinese IRB approval letter will be submitted to UCSF CHR before the study in China starts. The Subjects will have a 50/50 chance of being randomly assigned to each of the study groups. The first visit for saliva sample, baseline dental examination, QLF imaging, dental prophylaxis and

foam treatment will take about 1 hour. The following seven foam treatments will take about 10 minutes each time. The saliva sample visit at 1 month after completion of 1st foam treatments will take about 10 minutes. The final saliva and dental examination visit will take about 45 minuets. A total of about 3 hours will be involved for completion of the study. The dental examination at baseline and 1 year follow-up will be done by the addition of bitewing radiographs. Annual bitewing radiographs are a part of standard dental care for children with active dental decay. If there are no radiographs within 6 months available, the study will pay for the radiographs of the 1 year follow-up examination. One set of the radiographs will be provided to the patients' charts for their regular dentist and the other set will be kept with the subject's study charts.

D. Will any interviews, questionnaires, surveys or focus groups be conducted for the study? If "Yes," please list any standard instruments used for this study and attach any non-standard instruments.	[X]Yes [ ]No

Dental examination will be performed at baseline and 1 year after enrollment at the UCSF Predoctoral Dental Clinic with the standard dental setting and dental examination kit including a dental explorer and mirror. The dmfs/DMFS scores will be recorded using the standard recording form. QLF images will be taken after the initial and final dental exam, using QLF by Inspektor Pro (Omnii Oral Pharmaceuticals, USA). The Inspektor Pro QLF is a FDA approved non-invasive imaging instrument for early dental decay detection that uses filtered visible to induce fluorescence from the underlying dentin and the interference of this by a carious lesion is quantitatively determined. A brief questionnaire about their diet and oral care will be handed out at initial visit.
The patient will receive four weekly applications of either 2% NaF foam(control) or 10% povidone iodine plus 2% NaF foam(intervention) treatments consecutively in the first four weeks after enrollment. A dental prophylaxis will be performed immediately before the first foam treatment. The treatment will be repeated at the sixth month after enrollment. All treatments will be conducted in a regular pediatric dental setting. The subjects will be asked to bite on a commercial tray filled with either kind of the foam for 4 minutes and the excess foam will be removed by suction. The children will be asked not to rinse or eat for at least 30 minutes after the treatment.

The subjects will also be asked to chew on a piece of parafilm wax and spit into a sterile tube for 2ml of saliva at baseline, then 1 month, 5month and 1year after the first four foam treatments. The risk for this procedure is minor.

E. Will subjects undergo any study procedures or tests off-site by non-UCSF personnel? If "Yes," please explain.	[ ]Yes [X]No

F. Will subjects or their health care provider be given the results of any experimental tests that are performed for the study? If "Yes," please describe the tests, provide a rationale for providing subjects with the experimental test results and explain what, how and by whom subjects and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care decisions.	[X]Yes [ ]No

The guardians and subjects will be asked if they would like to know their salivary cariogenic bacterial levels at baseline and final visit after they complete the study. If they do want to know, a letter will be drafted to send them the results by the investigator in charge. The bacterial challenge category using the criteria previously devised by Dr. Featherstone will be used to interpret whether each subject is in high, medium or low bacterial challenge category. Patients will be encouraged to discuss their concerns and/or oral care plans with the investigators.

PART 4: ALTERNATIVES

A. Describe the alternatives to study participation that are available to prospective subjects.

Subjects who choose not to participate will continue to receive normal dental care in the appropriate clinic.

B. Is study drug or treatment available off-study? If "Yes," discuss this in the consent form.	[ ]Yes [X]No [ ]N/A

PART 5: RISKS AND BENEFITS

A. Risks and Discomforts:

1. Describe the risks and discomforts of any investigational or approved drugs, devices and procedures being used or assigned for study purposes. Describe the expected frequency of particular side effects. If subjects are restricted from receiving standard therapies during the study, please also describe the risks of those restrictions.

The 2% NaF foam has been approved to be used in children for caries prevention in U.S.A. 10% Povidone iodine is approved for intraoral use in the US. The risks resulting from saliva collection are minimal, although a small percentage of the population shows allergic reaction to iodine. According to our previous studies and those of other investigators, the oral use of povidone iodine has not been associated with any risks or discomforts above and beyond that associated with routine dental care.

QLF images will be taken after the initial and final dental exam, using an Inspektor Pro QLF device (Omnii Oral Pharmaceuticals, USA). The Inspektor Pro QLF is a FDA approved non-invasive imaging instrument for early dental decay detection. The risk of the QLF imaging is minimal.

2. Describe the steps you have taken to minimize the risks/discomforts to subjects (e.g., stopping rules, special monitoring):

Discomfort or delayed side-effects of either the fluoride or the povidone iodine plus fluoride foam will be built into the recording sheets for treatment and follow-up visits. The investigator in charge of the treatment and follow-up visit will ensure that this information is recorded and will report any side-effect to the principal investigator. The treatment will be stopped for any subjects with complications due to the treatment. Any side-effect related to the study treatment will be reported to CHR on time as required. A contact phone number of the responsible clinical investigator will be given to the patient for their questions and concerns about the treatment.

The annual bitewing x-ray is a part of your child's standard dental care. The study will pay the one year follow-up x-rays if they are not available. We will use the existing x-rays within 6 months and no new x-rays will be taken. The amount of radiation the child will be exposed to is relatively small. These doses of radiation could be potentially harmful, but the risks are so small that

they are difficult to measure.

B. Data and Safety Monitoring Plan:

The guidelines for a Data and Safety Monitoring Plan state that the degree of monitoring should be commensurate with the risk. Because the risk of adverse events related to the study is minimal and because we will take appropriate measures to ensure confidentiality the study will not require a Data Safety Monitoring Board. This study is a small scale pilot study to test if the combination of the two approved drugs, namely 2% sodium fluoride and 10% povidone iodine in a foam delivery system, will reduce the cariogenic bacteria and enhance resistance of the teeth to demineralization, thereby preventing future tooth decay in children at high risk. However we will conduct our own monitoring according to recognized procedures to prepare for and respond to any adverse events.

C. Confidentiality and Privacy: Describe the consequences to subjects of a loss of privacy (e.g., risks to reputation, insurability, other social risks):

Participation in research may involve a loss of privacy; however, the research records will be handled confidentially. All records will be coded, and kept in locked files so that only the study investigators have access to them. No individual identities will be used in any reports or publications resulting from this study.

1. Identifiers: Please indicate all identifiers that may be included in the research records for the study. Check all that apply.

[X] Names	[ ] Social Security Numbers	[ ] Device identifiers/Serial numbers
[X] Dates	[ ] Medical record numbers	[ ] Web URLs
[X] Postal address	[ ] Health plan numbers	[ ] IP address numbers
[X] Phone numbers	[ ] Account numbers	[ ] Biometric identifiers
[ ] Fax numbers	[ ] License/Certificate numbers	[ ] Facial

Photos/Images
[ ] Email address [ ] Vehicle id numbers [ ] Any other unique identifier
[ ] None of the 18 identifiers listed above

2. Determining Whether HIPAA Regulations Apply to This Study: Please answer the questions below for the identifiers marked in the above section. Check all that apply:	
Are study data: [ ] Derived from a medical record? *Please identify source*: [X] Added to the hospital or clinical medical record? [X] Created or collected as part of health care? [ ] Used to make health care decisions?	HIPAA regulations apply. The identifiers marked in section C.1 are PHI.
[X] Obtained from the subject, including interviews, questionnaires? [ ] Obtained from a foreign country or countries only? [ ] Obtained from records open to the public? [ ] Obtained from existing research records? [ ] None of the above.	HIPAA regulations do not apply. The identifiers marked section C.1 are not PHI.
If HIPAA regulations apply, you are required to obtain individual subject authorization or a CHR-approved waiver of authorization, or both, to be allowed access to medical records. For the VA, use the SFVAMC authorization. (The one exception to these requirements is the use of a Limited Data Set along with a Data Use Agreement.)	
3. Use and Disclosure of Personal Health Information: Please indicate to whom or where you may disclose any of the identifiers listed above as part of the study process. Check all that apply:	
[X] We do not plan to share any of the personally identifying information listed above outside the research team.	

[ ] The subject's medical record
[ ] The study sponsor: *please indicate*:
[ ] The US Food & Drug Administration (FDA)
[ ] Others: *please indicate*:
[ ] A Foreign Country or Countries

4. Data Security: Please indicate how study data are kept secure. Check all that apply:

[ ] Data are coded; data key is destroyed at end of study or *provide date*:
[X] Data are coded; data key is kept separately and securely
[X] Data are kept in locked file cabinet [X] Electronic data are protected with a password
[X] Data are kept in locked office or suite [ ] Data are stored on a secure network

5. Describe any additional steps taken to assure that identities of subjects and any of their health information which is protected under the law is kept confidential. If video or audio recordings will be made as part of the study, <u>disposition of these recordings</u> should be addressed here and in the consent form.

6. <u>Reportable Information</u>: Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse) or ethically requires action (e.g., suicidal ideation)? If "Yes," please explain below and include a discussion of the reporting requirements in the consent form.	[ ]Yes [X]No

D. <u>Benefits</u>: 1. Are there potential direct benefits to study subjects? If "Yes," please describe below.	[X]Yes [ ]No

The subjects recruited into the study will be at high risk of further tooth decay. They will get two free oral prophylaxis (about $60 each time) and eight fluoride

foam treatments (about $50 each time), which will help to them to reduce the risk for future decay. They will also get a free set of x-ray bite wings at the end of the study, which is worth $45. Besides, the results of the bacterial analysis will be available to the subjects by the end of the study, which indicates the subject's risk for future dental decay. It is hoped that the study will determine that the proposed regimen is a practical antibacterial treatment to reduce tooth decay causing bacteria and generate a new treatment regimen to prevent caries in children at high risk.

2. What are the potential benefits to society?

Potentially a PVP-I/F combined foam could be applied to the teeth of children at appropriate intervals throughout a year, reducing both the MS and LB bacterial challenge, enhancing remineralization, and markedly reducing or even eliminating new caries formation. The advantage expected by using this antibacterial/fluoride combination in a flavored foam form is improved taste and easier application for routine treatment, which may broaden its future use in dental practice or home dental care for caries prevention in children with high caries risk.

If the hypothesis is proven, it will indicate that PVP-IF foam is a convenient antibacterial treatment as a strategy in caries prevention for children in U.S.A. and beyond, especially for children with high caries risk from socioeconomically disadvantaged families.

E. Risk/Benefit Analysis: How do the benefits of the study outweigh the risks to subjects?

The subjects in the control group could get benefits from the study by 2 free prophylaxes and fluoride treatments, which will help them to improve oral hygiene and tooth resistance to decay. The subjects in the intervention group will get 2 free prophylaxes and PVPI/F foam treatment, which will have at least equivalent effect to fluoride foam treatment if it is not better, based on our preliminary study. The subjects will also get a free set of bite-wing x-rays of their tooth as a diagnostic tool for caries status assessment. Subjects in the control group will receive F foam treatment which is a part of routine care at UCSF for high caries risk children. Subjects in the intervention group will

receive PVP-IF treatment. PVPI has been approved to be used in oral cavity topically. The complication for PVPI is very rare although there are a small number of individuals who are allergic to iodine. Precautions are built into the recording system and the treatment will be stopped immediately and reported to CHR if any complication happens. The patient will be referred to the appropriate physician for treatment. The risk from other research procedures, such as saliva sampling, is minor. Therefore, the benefits to the subjects outweigh the risks in the study.

PART 6: SUBJECT INFORMATION

A. Number of Subjects:	
1. How many subjects will be enrolled at UCSF and affiliated institutions?	60
2. How many subjects will be enrolled at all sites (i.e., if multicenter study)?	120
3. How many people do you estimate you will need to consent and screen here (but not necessarily enroll) to get the needed subjects?	120

B. Types of Subjects: Check all that apply. Click on links for additional instructions.	
[X]	Minors.....Attach "Inclusion of Minors" Supplement
[ ]	Subjects unable to consent Attach Surrogate Consent or Emergency Waiver of Consent Supplement
[ ]	Subjects unable to read or speak English
[ ]	Pregnant Women
[ ]	Fetuses
[ ]	Neonates
[ ]	Prisoners .Attach "Inclusion of Prisoners" Supplement
[ ]	Inpatients
[X]	Outpatients

[ ]	Healthy Volunteers
[ ]	Staff of UCSF/affiliated institution

C. Eligibility Criteria:

1. General description of subject population(s):

A total of 60 healthy 6-9 year olds with 1-5 frank caries lesions (who assent and their parents give consent) will be enrolled in the study at each site, randomized to 30 per each of the test and control groups. Eligible children who reside within a 25 mile radius and will be randomly assigned to one of the two study groups.

2. Inclusion Criteria:

Children: a) 6-9 years old, b) registered patients at UCSF Predoctoral Pediatric Dental Clinic or Postgraduate Pediatric Dental Clinic, c) have 1-5 frank caries lesions, d) resident within a 25 miles radius of San Francisco.

3. Exclusion Criteria:

children: a).with serious chronic systemic or periodontal diseases; b).with medicines taken within the past 3 months that might affect oral flora; c).with a dry mouth or difficulty spitting

D. How (chart review, additional tests/exams for study purposes), when and by whom will eligibility be determined?

A dental examination of dmfs/DMFS will be conducted by Dr. Ling Zhan. She is a postgraduate researcher in the Department of Preventive and Restorative Dental Sciences at the UCSF. She is a trained dentist with over 10 years of clinical practice experience. Her research career has been focused on microbiology aspects of caries and caries prevention. She has worked closely with Dr. Jane Weintraub in one NIH funded caries management study and has been trained as an examiner for dmfs/DMFS scores using WHO criteria.
All prophylaxis and foam treatments will be done at PDPDC or PGPDC by one postgraduate dental resident (Dr. Purvi Zavery) under supervision of Dr. Pamela Den Besten, who is the chair and professor of the Division of Pediatric Dentistry. The final bite-wing x-rays will be also taken by the resident.

E. Are there any inclusion or exclusion criteria based on [ ]Yes [

gender, *race* or *ethnicity*? If "Yes," please explain the nature and rationale for the restrictions below.	X]No

PART 7: RECRUITMENT

A. Please review CHR Recruitment Guidelines for more information about acceptable recruitment methods. Note that all advertisements, whether posted or broadcast, and all correspondence used for purposes of recruitment require CHR review and approval before they are used. Check all that apply:	
[X]	Study investigators recruit their own patients directly and/or nurses or staff working with researchers approach patients. *Please explain in Section B.*
[ ]	Study investigators send a CHR-approved letter to colleagues asking for referrals of eligible patients interested in the study. The investigators may provide the referring physicians a CHR-approved Information Sheet about the study to give to the patients. If interested, the patient will contact the PI. Or, with documented permission from the patient, the PI may be allowed to talk directly with patients about enrollment. *Attach letter for review.*
[ ]	Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing. *Attach letter for review.*
[X]	Advertisements, notices, and/or media used to recruit subjects. The CHR must first approve the text of these, and interested subjects will initiate contact with study investigators. *Attach ads, notices, or media text for review. In Section B, please explain where ads will be posted.*
[ ]	Study investigators request a Waiver of Consent/Authorization for recruitment purposes. This waiver is an exception to the policy but may be requested in circumstances such as:

[ ] [ ] [ ]	Minimal risk studies in which subjects will not be contacted (i.e., chart review only); Review of charts is needed to identify prospective subjects who will then be contacted. (Explain in Waiver form); Large-scale epidemiological studies and/or other population-based studies when subjects may be contacted by someone other than personal physician. (Explain in Waiver form.)
[ ]	Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study. *Please explain in Section B.*
[ ]	Study investigators list the study on the UCSF Clinical Trials Seeking Volunteers web page or a similarly managed web site. Interested subjects initiate contact with investigators.
[ ]	Study investigators recruit potential subjects who are unknown to them. Examples include snowball sampling, use of social networks, direct approach in public situations, random digit dialing. *Please explain in Section B.*

B. Provide detail in the space below (*i.e., how, when, where and by whom are potential subjects approached?*).

The students, faculty and staff in the PDPDC and PGPDC will be informed about the study and be asked to refer potentially qualified patients to the study investigators or give the patient the study contact phone number for them to contact the investigators. The investigator will approach the patient's guardian when the patients come for their regular treatment and ask if they are interested in the study. A screening appointment will be scheduled if the patient and their guardian are interested.

Fliers about the study with contact information will also be posted in the UCSF School of Dentistry Clinics to recruit subjects.

PART 8: INFORMED CONSENT PROCESS

A. Check all that apply:

[X] Signed consent will be obtained from subjects and/or parents (if subjects are minors),

[ ] Verbal consent will be obtained from subjects, using an

[ ] Information sheet (attach)

[ ] Script (attach)

[ ] Signed consent will be obtained from surrogates Attach Surrogate Consent Supplement

[ ] Informed consent will not be obtained. Attach either the Waiver of Consent/Authorization or the Emergency Waiver of Consent Supplement as appropriate.

B. In the space below, describe *how*, *where*, *when* and *by whom* informed consent will be obtained. How much time will prospective subjects be given to consider study participation? If special subject populations will be included, be sure to describe any additional plans for obtaining consent from particular populations. Justify any plans to use verbal consent instead of signed consent.

Informed consent from subject's guardian and assent from the subject will be obtained by Dr. Den Besten, Dr. Ling Zhan and/or the Pediatric Dental resident who will work on the study, at the scheduled screening visit in PDPDC or PGPDC. The above named doctors will discuss the procedures, benefits, risks and rights of the subject in the study and answer questions. The subjects will have 30 minutes to decide whether they want to participate in the study. If they can not decide in 30 min, they will always be welcome to call back to schedule a visit for their enrollment while the study recruitment is going on.

C. How will you make sure subjects understand the information provided to them?

The investigator will ask the subject to repeat the outline of the study procedures and their benefits and risks to make sure they understand the provided information.

PART 9: FINANCIAL CONSIDERATIONS

A. Payments to Subjects: 1. Will subjects receive payments or gifts for study participation? If "Yes," please review CHR Subject Payment Guidelines and complete the following:		[X]Yes [ ]No
2. Payments will be (check all that apply):	[X] Cash [ ] Check [ ] Other (describe below)	
3. Please describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.		

Each child will receive a $10 gift card and their parent/guardian will receive $10 cash for each of the treatment or saliva visits. Both of the child and his/her parent/guardian will get $4 per visit cash bonus at the final visit that will be $40 cash for each of them if they complete all ten study visits. That is a total of $100 gift card and $40 cash reimbursement for each subject and $140 cash for their parent if they complete the study. The reimbursements to the parents/guardians are calculated to cover their parking and transportation expenses for the visit. We added the cash bonus at the final visit to show our appreciation for their participation in the study and encourage them to complete the study.

The subjects and the parents in China will receive 20 Chinese Yuan gift card and the parent/guardian will receive 20 Chinese Yuan for completion of each four weekly treatment visit at the beginning and the 6th month after enrollment. The child will receive a 10 Chinese Yuan gift card and their parent/guardian will receive 10 Chinese Yuan for the 1 month saliva sample visit and the 12 month final visit. The subject and their guardian will get 4 Chinese Yuan per visit cash bonus at the final visit that will be 40 Yuan cash bonus for each of them if they complete all study visits. That will be a total of 200 Chinese Yuan reimbursement for the child and their parent/guardian if they complete the study.

B. Costs to Subjects: Will subjects or their insurance be charged for any study procedures? If "Yes," describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.	[ ]Yes [X]No

C. Treatment and Compensation for Injury: The investigators are familiar with and will follow the University of California policy and (if applicable) Veteran's Affairs policy regarding treatment and compensation for injury. If subjects are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, by the Department of Veteran's Affairs (for subjects eligible for veteran's benefits, if the SF VAMC is a study site), or by the study sponsor, if any, depending on a number of factors. The University does not normally provide any other form of compensation for injury.

PART 10: BIBLIOGRAPHY

LITERATURE CITED:

1. Kaste LM SR, Oldakowski RJ, Brunelle JA, Winn DM, Brown LJ. Coronal caries in the primary and permanent dentition of children and adolescents 1-17 years of age: United States, 1988-1991. J. Dent. Res. 1996;75:631-641

2. Macek MD, Heller KE, Selwitz RH and Manz MC. Is 75 percent of dental caries really found in 25 percent of the population? J Public Health Dent 2004;64:20-5

3. Featherstone J. The science and practice of caries prevention. J Am Dent Assoc 2000;131:887-899

4. Napimoga MH, Kamiya RU, Rosa RT, et al. Genotypic diversity and virulence traits of Streptococcus mutans in caries-free and caries-active individuals. J Med Microbiol 2004;53:697-703

5. KHOO MA, DENBESTEN PK, FUJINO T, et al. MS Genetic Diversity and Cariogenic Bacterial Levels in ECC Subjects. J Dent Res 2004;83:A3431
6. Zhan L, Hoover C, Li W and Featherstone JDB. Genetic Diversity of Mutans Streptococci in High Caries Risk People. J. Dent Res 2002;81:A-351
7. Caufield PW, Gibbons RJ. Suppression of Streptococcus mutans in the mouths of humans by a dental prophylaxis and topically-applied iodine. J Dent Res 1979;58:1317-26
8. Lopez L, Berkowitz R, Spiekerman C and Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries: a follow-up report. Pediatr Dent 2002;24:204-6
9. Zhan L, DenBesten PK, Gansky SA, Hoover CI, Fujino T and Featherstone JD. Povidone-iodine as an oral antiseptic in children with early childhood caries. Caries Res 2003;37:272
10. Hoover C, Weintraub JA, Gansky SA, White JM, Wilson RS and Featherstone JD. Effect of a Caries Management Regimen on Cariogenic Bacterial Population. J. Dent Res 2004;83:A0779
11. Wall-Manning GM, Sissons CH, Anderson SA, Lee M. Checkerboard DNA-DNA hybirdisation technology focused on the analysis of gram-positive cariogenic bacteria. J Microbiol Methods 2002;51:301-311.

PART 11: ATTACHMENTS

Please list Attachments, Supplements and Appendices	Version number(s) or date(s)

consent form
assent form
flyer
Form A. Baseline visit
Baseline dmfs/DMFS record form
Baseline questionnaire
Form B. 1 week- 2 month follow-up

visit record form

Form C. 6 month follow-up record form

Form D. Final visit record form

Final dmfs/DMFS record form

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT

Effectiveness of Specific Antimicrobial Treatment against Bacteria that cause Dental Decay in Children in Pacific Rim Countries

A. PURPOSE AND BACKGROUND

Dr. John Featherstone, Dr. Ling Zhan from the Department of Preventive and Restorative Dental Sciences and Dr. Den Besten from the Division of Pediatric Dentistry are conducting a study to investigate using Povidone Iodine Foam as an antibacterial treatment to reduce new tooth decay in children with high risk. This study is being funded by a Pacific Rim Grant from the University of California, and a grant from the International Association for Dental Research.

Your child is being asked to participate in this study because he/she is at high risk for tooth decay and is 6 to 9 years old.

B. PROCEDURES

If you agree to have your child in this study, the following will happen to your child:

1. Your child will have a 50/50 chance (like flipping a coin) of being placed in one of two groups. Neither your doctor nor you will make the choice, so that bias in the study is reduced. The two groups are (a) fluoride foam (control group) or (b) povidone iodine/fluoride (treatment group). He/she will be scheduled for an initial visit. A dental examination will be done to record his/her tooth decay status. Pictures of his/her back teeth will be taken with a intra oral camera with a blue light shine on the teeth. He/she will be asked to chew on a piece of parafilm wax and spit into a sterile tube and give 2ml of saliva. The saliva will be used to test the tooth decay causing bacteria. He/she will receive a dental prophylaxis (professional tooth cleaning). Then he/she will be asked to bite on a tray filled with fluoride foam (control group) or povidone iodine/fluoride foam (treatment group) for 4 minutes based on

which group he/she will be randomly assigned to. He/she will be asked not to rinse or eat for 30 minutes after the treatment. The initial visit will take about 1 hour.

2. Your child will be asked to come back for three more foam treatments, one each week for the next 3 weeks after the initial treatment. Each of these foam treatments will take about 10 minutes.
3. Your child will be asked to come back one month after the last foam treatment and spit into a sterile tube to give 2 ml of saliva. The visit will take about 10 minutes.
4. Four months later, he/she will be asked to come back to repeat the foam treatment once a week consecutively for four weeks. He/she will spit into a tube to give 2ml of saliva and receive a prophylaxis before the first foam treatment. Each visit will take about 10 minutes.
5. One year after enrollment, your child will be asked to come back for the final visit. A set of bitewing x-rays of the teeth will be taken if there are no existing bitewing x-rays taken within 6 months. One set of the x-rays will be available for your child's dentist. He/she will be asked to spit to give 2ml of saliva.He/she will also receive a dental examination and pictures on his/her back teeth will be taken again, using an intra oral camera with blue light. The visit will take about 45 minutes.
6. Arrangement will be also made in the UCSF Pediatric Dental Clinic to have all cavities of your child fixed within 6 month after he/she enters the study. Payment for this routine treatment will come either from your dental insurance or from you.

Participation in the study will take a total of about 2 hours 50 minutes over a period of 1 year with a total of 10 visits.

C. RISKS/DISCOMFORTS

The 2% NaF foam is a standard treatment that is approved for use in children for caries (tooth decay) prevention in U.S.A. The 10% Povidone iodine antibacterial treatment is approved for use in the mouth in the US. The risks resulting from saliva collection are minimal, although a small percentage of the population shows allergic reaction to iodine. According to previous studies, the oral use of

povidone iodine has not been associated with any risks or discomforts above and beyond that associated with routine dental care.

The annual bitewing x-rays are a part of your child's standard dental care. The study will pay for the one year follow-up x-rays if they are not available. We will use any existing x-rays taken within 6 months and no new x-rays will be taken. The amount of radiation your child will be exposed to is relatively small. These doses of radiation could be potentially harmful, but the risks are so small that they are difficult to measure. If your child has had a lot of x-rays already, you should discuss this with the investigator.

Randomization: Your child will be randomly assigned to a treatment program by 50/50 chance. The treatment your child receives may prove to be less effective or to have more side effects than the other study treatment or than other available treatments. This will not be known until after the study is completed and the data has been analyzed.

Confidentiality: Participation in research may involve a loss of privacy, but information about you will be handled as confidentially as possible. Records will be coded, and kept in locked files so that only the study investigators have access to them. No individual identities will be used in any reports or publications resulting from this study.

Treatment and Compensation for Injury:

If your child is injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at (415) 476-1814.

D. BENEFITS

The potential benefit to your child is that the treatment he/she receives may prove to be more effective than the other study treatment or than other available treatments, although this cannot be guaranteed. It is hoped that the

information gained from the study will help to prevent or reverse dental decay in children at high risk in the future. Further, the results of the bacterial analysis will be available to you at the end of the study. This information will be explained to you as indicators of your child's risk for future dental decay. It is hoped that the study will determine that the proposed regimen is a practical antibacterial treatment to reduce the number of tooth decay causing bacteria. If successful this will be a new treatment for preventing or reversing dental decay in children at high risk.

E. ALTERNATIVES

Your child and you may choose not to participate in this study at any time and no further exam, treatment, or saliva samples will be taken but you will continue to receive normal dental care in the appropriate clinic.

F. COSTS

You will only pay for the usual treatments that are needed to fix your child's tooth decays as a part of their routine dental care. Your child will not be charged for any exam, fluoride or antibacterial treatments or saliva collection that are part of the study.

G. PAYMENT

You will receive $10 cash and your child will receive a $10 gift card for each study visit. You and your children will also get $4 cash bonus for each visit at the final visit. That is a total of $140 for each of you if your child completes the study.

H. QUESTIONS

This study has been explained to you by Dr. John Featherstone, or Dr. Ling Zhan, or the person who signed below and your questions were answered. If you have any other questions about the study, you may call Dr. John Featherstone at (415)476-0456, or Dr. Ling Zhan at (415) 476-0921.

I. CONSENT

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate, you should sign below, and you will be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about your child.

The person being considered for this study is unable to consent for himself/herself because he or she is a minor. You have been asked to give your permission to include your child in this study. You know of no reason why he/she would refuse were it possible to do so now.

_____ _____

Date Parent/Legal Guardian's Signature

_____ _____

Date Person Obtaining Consent

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
ASSENT TO BE A RESEARCH SUBJECT

Effectiveness of Specific Antimicrobial Treatment against Bacteria that cause Dental Decay in Children in Pacific Rim Countries

What is this study about?

Dr. John Featherstone and Dr. Ling Zhan are doing an experiment to see if a special tooth foam will help to stop cavities better than regular tooth foam.

What will happen to you if you are in the experiment?

1. First the dentist will look at your teeth. A picture of your back teeth will be take by a mini camera with blue light. You will chew on a piece of wax and spit into a cup until a teaspoon of spit is collected.
2. Your teeth will be cleaned and then you will bite into a tray that is filled with fruit flavored foam for 4 minutes.
3. The extra foam in your mouth will be removed with a suction straw.
4. You will come back 3 more times in the next 3 weeks to bite the foam trays. One month later, you will come back to chew on wax and spit into a cup again.
5. Four months later, you will come back to chew on wax and spit into a cup for one teaspoon of spit. Your teeth will be cleaned and you will bite the foam trays once a week for four times.
6. Then you will come back 5 months later to have the dentist look at your teeth and take the picture of your back teeth with the mini camera. You will chew on a piece of wax and spit into a cup.

Will any parts of the experiment hurt?

There is no part of this experiment that will hurt. It is like your regular check up with your dentist.

Will the study help you get better?

Yes, we hope the study will find new ways to keep children from getting new tooth decay in future. The tooth cleaning and fluoride foam treatments you will receive in the study are going to help you keep your teeth clean and strong.

What if you have questions?

You can ask Dr. John Featherstone or Dr. Ling Zhan or their friends any questions you have about the experiment. You can ask your questions now or later, any time you like.

What are your choices?
You will be in this experiment only if you want to. You will not be in it if you don't want to. If you decide to be in this experiment and you change your mind later, that is okay too. You just have to tell the dentist and then you can stop. No body will get mad at you if you don't want to be in this experiment.

If you are in the experiment, you will get a $10 gift card for each study visit and you will get $4 bonus for each visit at the final visit. If you finish all 10 study visit, you can get $100 in gift cards and $40 cash.

If you want to be in this experiment, please sign your name on the line at the bottom of this paper.

Date Signature of Child

UCSF COMMITTEE ON HUMAN RESEARCH Please date form: __
APPLICATION SUPPLEMENT
(BETA VERSION)

INCLUSION OF MINORS

Principal Investigator on CHR Application:	CHR # (if known):
John Featherstone	
Study Title (may not exceed 500 characters):	
Effectiveness of Specific Antimicrobial Treatment Against Bacteria that Cause Dental Decay in Children in Pacific Rim Countries	

Age Range Please specify the eligible age range for minors in this study:

six to nine years old.

45 CFR 46, Subpart D: Minors Research on minors must fall under one of the following categories. Please check all that apply:	
]	Minimal Risk (45 CFR 46.404). The risks (physical or emotional) are no greater than those encountered in daily life or during the performance of routine physical or psychological examinations or tests. Obtain the consent of one parent/legal guardian and the assent of the minor (if over 7 years of age).
X]	Greater than Minimal Risk (45 CFR 46.405) but presenting the prospect of direct benefit to the individual subject. Obtain the consent of one parent/legal guardian and the assent of the minor (if over 7 years of age).
]	Greater than Minimal Risk (45 CFR 46.406) (though only a minor increase over minimal risk) and no reasonable prospect of direct benefit to the individual subject, but likely to yield generalizable knowledge about the subject's disorder or condition. Obtain the consent of both parents/legal guardians and the assent of the minor (if over 7 years of age) unless one parent is

	deceased, unknown, incompetent, not reasonably available, or does not have legal responsibility for the custody of the minor.
[ ]	Research not otherwise approvable (45 CFR 46.407) which presents an opportunity to understand, prevent, or alleviate serious problems affecting the health or welfare of children. • Requires approval by the Secretary of the U. S. Department of Health and Human Services. • Requires consent of both parents/legal guardians.
If there is more than one group of minors being studied, e.g., patients and normal controls, and the groups fall into different risk/benefit classifications, explain the differences below, citing the applicable section of 45 CFR 46 for each group.	

Informed Consent and Minor's Assent
Please indicate below the consent and assent forms that will be used for this study.

[ ] Consent form addressed to the subject, for subjects 13 and older, with signature lines for subjects and parent(s)

[X] Simplified Assent form for subjects 7-12 years of age

[X] Consent form addressed to parent(s)

Waiver of parental consent (45 CFR 46.408(c)): The requirement for parental/guardian consent may be waived under limited circumstances (e.g., homeless youth, youth seeking health care which does not require parental consent) where parental/guardian permission is not a reasonable requirement to protect the subject if an appropriate mechanism for protecting the children is implemented and the waiver is consistent with Federal, State, or local law.

If you are requesting a waiver of parental/guardian consent, please explain why parental consent is not necessary and what other protections are in place to substitute for parental consent.

A Study to Prevent Tooth Cavities in Children

Recruiting Volunteers!

It is a one year study with fruit flavored antibiotic foam treatments.

- We need children age 6 - 9 years with 1 to 5 cavities
- The child will receive $100 gift certificate, $40 cash, two free tooth cleaning and a free set of tooth x-ray in one year.
- The parent will receive $140 cash.

For details of the study please contact:

Dr. Ling Zhan
Phone: (415) 476-0921
University of California, San Francisco
Department of Preventive and Restorative Dental Sciences
Division of Clinical General Dentistry

PVP-I Foam Study

Form A1 Subject's Contact Information and Ethinicity

Subject initial:_____. Study ID:PR_____/_____/_____.

Please fill in the following information (It will only be used for contact in this study):

Child's Name: _____

_____

First Last

Middle Initial

Child's Birth date: _____/_____/_____

MM DD YYYY

Child's gender: Male____ Female_____

Parent/guardian's Name:_____

First Last

Contact phone number: _____(day) _____ (night)

Contact Address:

Number Street Apt#

City State Zip Code

Information about you child's ethnicity:

Is your ethnic background Hispanic, Latino or other Spanish descent?

☐ No
☐ Yes

☐ Central American
☐ Cuban
☐ Mexican
☐ Puerto Rican
☐ South American
☐ Other Hispanic

Please select your racial background (you may select more than one):

☐ African-American / Black / Haitian
☐ American Indian / Native American / Alaskan Native
☐ Asian
☐ Bangladeshi
☐ Burmese/Myanmarese
☐ Korean
☐ Laotian

☐ Chinese ☐ Malaysian

☐ Filipino ☐ Pakistani

☐ Indian ☐ Thai

☐ Indonesian ☐ Vietnamese

☐ Japanese ☐ Other Asian

_____

☐ Caucasian / White / Middle Eastern

☐ Native Hawaiian / Pacific Islander

☐ Fijian ☐ Samoan

☐ Guamanian ☐ Tongan

☐ Hawaiian ☐ Other Pacific Islander _____

☐ Other _____

☐ Do not wish to respond

PVP-I Foam Study

Form A2 Subject Oral Care Survey

Subject initial: _____.

Study ID: PR_____/_____/_____.

Please answer the following questions about your child's oral health care habits as best as you can:

Child's Name: _____

First Last

Child's Birth date: _____/_____/_____.

MM DD YYYY

Child's gender: Male____ Female_____

1. How often does your child brush his/her teeth?

- □ Not every day
- □ 1 time each day
- □ 2 times each day

2. Does your child use fluoride toothpaste when he/she brushes his/her teeth?

- □ Yes
- □ No
- □ I do not know.

What kind of toothpaste does your child use? _____

3. How many times each day does your child eat or drink sweet snacks like soda, juice, cookies and candy etc.?

- □ Never
- □ 1 time each day
- □ 2 times each day
- □ 3 or more times each day

4. How many times did your child floss his/her teeth in the past week?

- □ Not at all
- □ One Time
- □ Two Times
- □ Three to six times
- □ Daily (seven times)
- □ More than seven times
- □ Don't know

5. Did your child see a dentist at least once a year in the past two years?

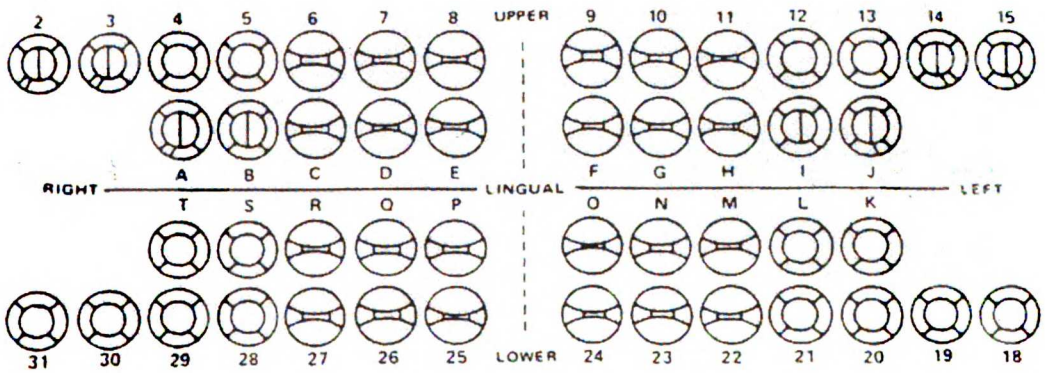
- □ Yes.
- □ No.

5. Would you like to know your child's test results at the end of the study?

- □ Yes
- □ No

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO SCHOOL OF DENTISTRY
PVP-I Foam Study Form A3 Baseline DMFS/dmfs record sheet
Subject's initials: _____. Study ID: PR_____/_____/_____.
Visit Date: ____/____/_____

Charting: Red=current decay, Blue= previous restorations



Comments:

_____

_____

_____

_____

_____

_____

Patient's Name _____ __	Date of visit: ____/____/____	Patient Study ID Number PR___/___/___

PROCEDURES			
Subject Qualification			
1. 1-5 active caries in past year?		Yes	No
2. No antibiotics within 2 weeks?		Yes	No
3. No medicine that will cause dry mouth?		Yes	No
4. No hepatitis and HIV etc. systemic disease.		Yes	No
5. Will stay in the Bay Area for another 1 year.		Yes	No
6. No significant developmental dental diseases.		Yes	No
Patient qualified for the study		Yes	No
Consent form signed by the patient?		Yes	No
Saliva sample collected? (1 tube, 2 ml in ≤ 4 minutes)		Yes	No
DMFS/dmfs exam done?		Yes	No
Oral prophylaxis done?		Yes	No
First Foam Treatment			
Randomization(check one group)	Group A _____ Group B ____		
Foam treatment done (record the foam group)	Foam _____		
Record any unpleasant feeling or complaint after the foam treatment. If yes, report it to Dr. John Featherstone.	a. None _____. b. unpleasant taste _____ c. staining _____ d. allergy _____ e. others _____		
Schedule the date for the second foam visit(include week day also): ____/____/_____(_____)			

Comments:_____
Investigator's Signature:

Patient's Initials _____	Patient treatment group: _____.	Patient Study ID Number PR___/___/___

Second Foam Treatment:	
Date of visit	____/____/_____.
Record any unpleasant feelings or complaints after the previous foam treatment. If yes, report it to Dr. John Featherstone.	a. None _____. b. unpleasant taste _____ c. staining _____ d. allergy _____ e. others _____
Foam treatment done? (record the foam group)	Group _____.
Record any unpleasant feelings or complaints after the present foam treatment. If yes, report it to Dr. John Featherstone.	f. None _____. a. unpleasant taste _____ b. staining _____ c. allergy _____ d. others _____
Schedule the date for the third foam visit(include week day also): ____/____/____(_____)	
Investigator's Signature:	

Third Foam Treatment	
Date of visit	____/____/_____.
Record any unpleasant feelings or complaints after the previous foam treatment. If yes, report it to Dr. John Featherstone.	a. None _____. b. unpleasant taste _____ c. staining _____ d. allergy _____ e. others _____
Foam treatment done? (record the foam group)	Group _____.
Record any unpleasant feelings or complaints after the present foam treatment. If yes, report it to Dr. John	a. None _____. b. unpleasant taste _____ c. staining _____ d. allergy _____ e. others _____

Featherstone.	
Schedule the date for the fourth foam visit(include week day also):____/___/____ (_____)	
Investigator's Signature:	

Fourth Foam Treatment	
Date of visit	____/____/_____.
Record any unpleasant feelings or complaints after the previous foam treatment. If yes, report it to Dr. John Featherstone.	a. None _____. b. unpleasant taste _____ c. staining _____ d. allergy _____ e. others _____
Foam treatment done? (record the foam group)	Group _____.
Record any unpleasant feelings or complaints after the present foam treatment. If yes, report it to Dr. John Featherstone.	a. None _____. b. unpleasant taste _____ c. staining _____ d. allergy _____ a. others _____
Schedule the date for 1 month saliva visit(include week day also): _____/_____/_____(_____)	
Investigator's Signature:	
1 month saliva sample	
Date of visit	____/____/_____.
Record any unpleasant feelings or complaints after the previous foam treatment. If yes, report it to Dr. John Featherstone.	a. None _____. a. unpleasant taste _____ b. staining _____ c. allergy _____ d. others _____
Saliva sample collected? (1 tube, 2 ml in $\leq$ 4 minutes)	Yes. ____
Schedule the date for 2nd set of foam treatment visit at 6th month (include week day also):	

_____/_____/_____(_____)
Does the patient have any change on their contact information: Yes. No. If "Yes", please update the patient contact information.
Investigator's Signature: