Title

A study on the effectiveness of povidone iodine foam against oral cariogenic bacteria in children with active caries

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Publication Date

2005

Peer reviewed|Thesis/dissertation

A STUDY ON THE EFFECTIVENESS OF POVIDONE IODINE FOAM AGAINST ORAL CARIOGENIC BACTERIA IN CHILDREN WITH ACTIVE CARIES

by
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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral Biology

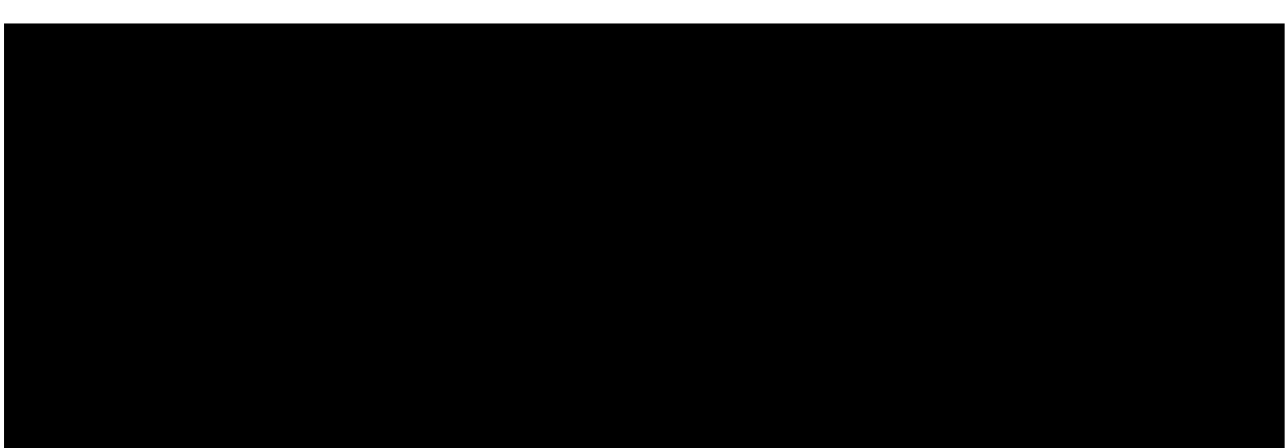
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



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DEDICATION

I would like to dedicate this paper to the special people in my life who have provided me with kindness and words of encouragement when I have needed them most. This paper is dedicated to my mom and dad, Rena and Ignacio Brambila, for always believing in me, and to my fiancé Marcelo Braga for making me believe in myself.

ACKNOWLEDGEMENTS

I would like to acknowledge with much gratitude the support and encouragement of my thesis committee. Thank you to Dr. Pamela Den Besten for her guidance and support throughout this study and during the writing of this thesis. My appreciation to Dr. John Featherstone for his all of his editing and constructive commentary of this paper. Thank you to Dr. Arthur Miller for his critique of this thesis. Also, my gratitude to Dr. Chuck Hoover for his critical review of the content and results sections. And lastly, thank you to Dr. Ling Zhan, for all her patience, encouragement, and dedication to every phase of this project.

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1. BACKGROUND

1.1 Epidemiology of Dental Caries

Dental caries, also known as tooth decay, is the pathologic process of tooth destruction by oral microorganisms that can affect individuals of all ages and all cultural, ethnic and socio-economic backgrounds. Newacheck (2000) reported dental care was the most common unmet health care need among children in the United States.[1] Dental caries is the single most common chronic disease of childhood, with a rate five times greater than that seen for the next most prevalent disease of childhood- asthma.[2] Because dental infections are common and usually non-life-threatening in nature, the significance of dental decay in overall human health has historically been minimized.[3]

Dental caries seems to be a sequestered disease, meaning that a few individuals possess a disproportionate amount of the overall decay. It has been estimated that approximately 25% of children and adolescents in the 5- to 17- year age range account for 80% of the caries in permanent teeth.[4-8] As children transition into adulthood, this disproportionate caries distribution changes slightly. After 17 years of age it is approximated that 40% of the population account for 80% of caries.[4,5,8,9] This suggests that the population of individuals susceptible to dental decay continues to expand with increasing age.

Although overall dental caries prevalence and severity has been notably reduced in several Western world countries over the past couple of decades, dental health

continues to be a major health concern in the United States. The Third National Health and Nutrition Examination Survey (NHANES III)-Phase I, collected data from 1988 to 1994 which indicated that 50% of 5-8 year old children in the United States had experienced caries in the primary dentition.[10] NHANES III also supported the notion that caries experience increased with age. From the data assembled from NHANES III, it was determined that 67% of 12-17 year olds had experienced dental decay in the permanent dentition and that 94% of dentate adults have experienced coronal (enamel) caries at some point in their lives.[4,5,8-10] It is evident from numerous studies that dental decay continues to affect individuals through childhood and beyond.

Despite the fact that some individuals remain highly susceptible to dental decay, the generalized overall decrease in caries prevalence experienced by a large percentage of the population cannot be associated with any single event. It is a common belief that this observation is due most notably to the incorporation of fluoride into routines of daily living. With the inclusion of fluoride in toothpastes, mouthrinses, and selected water supplies since the mid- 1970's, a decline in overall caries levels in the United States and several other Western world countries has been observed.[11,12] Unfortunately, the beneficial effects often credited to fluoride seem to have reached a plateau since the 1990's.[9]

Untreated dental decay continues to persist regardless of thorough and consistent oral hygiene instructions and cutting-edge restorative techniques and continual development of new and improved dental materials. Based on data collected from 1993 to 1994 concerning the dental health of children in California, it was found that 55% of children 6-8 years old had untreated tooth decay.[13,14] The treatment of dental infections is

costly because it is rendered on a symptomatic basis. It is usually not until dental decay has caused radiographic or frank tooth destruction, possibly accompanied by pain, that the infected portion of the tooth is excised and replaced with a stable, bioinert dental material.

Despite various caries preventive methodologies and procedures currently in use, a portion of the population continues to remain at high risk of developing caries. Unfortunately routine treatment protocols do not include a viable preventive approach based upon the control of the bacterial factors involved in decay.[15] These high-risk individuals are most likely in need of added preventive measures such as antibacterial treatments in order to reduce their cariogenic bacterial loading and consequently decrease the intensity of their caries challenge.

Currently dental caries is one of the most common infectious diseases inflicting humans. Due to issues related to access to care, such as finances, location of providers, and patient education, dental decay remains untreated in many underdeveloped countries. Untreated dental decay leads to considerable suffering that is often alleviated only by the loss or extraction of the infected tooth.[16] The impact of dental caries extends beyond the boundaries of health: the resulting oral pain and chronic discomfort may affect speech, eating, sleeping, swallowing, breathing, and the altered appearance it causes to any visible teeth may undermine self-image, self-esteem, and social acceptance.

1.2 The Caries Process

Dental caries is a multifactorial, transmissible, bacterial disease involving an imbalance between protective and pathological factors.[17] There are three requirements

necessary for the initiation and progression of a dental lesion. In order for breakdown of tooth structure to occur, there must be a susceptible host, cariogenic microorganisms, and the presence of a suitable substrate for a sufficient length of time.[17]

Enamel, a mineral that is the hardest of all human tissues, is comprised of carbonated hydroxyapatite and serves as the initiation site of dental caries. The caries process begins with the development of plaque- a sticky film consisting of a mixture of bacteria, bacterial byproducts, salivary components and fermentable carbohydrates, which forms on the surface of the tooth (enamel). Cariogenic bacteria present on the tooth surface within the plaque have the capability to metabolize available fermentable carbohydrates, and as a result produce lactic and other acids. The resultant acid byproducts, when given the opportunity to interact with the tooth surface over a period of time, may then dissolve the calcium phosphate mineral of the carbonated hydroxyapatite tooth enamel, and cause a non-cavitated white-spot lesion within the enamel in a process known as demineralization.[17-19]

If pathological factors such as acidogenic bacteria, reduced salivary function, and high frequency of fermentable carbohydrate ingestion persist within the oral environment, the sub-surface enamel demineralization will progress further, following along the radial course of the enamel prisms to the dento-enamel junction. At this point a cavitation results in the enamel.[20] Once caries reaches the dento-enamel junction, it spreads laterally and centrally into the dentin. If it continues to remain unchecked, then the lesion may continue to advance and penetrate into the pulp. Pulpal insult can lead to the loss of tooth vitality and eventually can lead to the pathogenic loss of the bacterially infected tooth.

Early demineralization (non-cavitated white-spot lesions) may be halted, allowing for the re-incorporation of lost minerals into the hydroxyapatite structure of the enamel in a process known as remineralization. Increasing the presence of protective factors- such as salivary components (immunoglobulins, calcium phosphate, and protein), salivary flow, topical fluoride, and antibacterials make it possible to remineralize these early lesions.[21] If circumstances allow for remineralization to proceed in the presence of fluoride, the restored tooth structure may consist of fluorhydroxyapatite, an improved structure that is stronger and more resistant to acid than the original structure.[21] Although the formerly decalcified area of the tooth may be as strong or stronger than its original formation, a chalky, dull, white coloring may persevere in the specific area that experienced acid challenge.

1.3 Cariogenic Bacteria

While many oral microorganisms may participate in the demineralization and cavitation of enamel and dentin, there are specific bacteria that demonstrate a higher carigenicity than other oral flora. Numerous studies have documented that specific bacteria exert a strong influence on the caries process. The most influential cariogenic bacteria are mutans streptococci (mainly *S. mutans* and *S. sobrinus* in humans) and lactobacilli. They are able to contribute to the caries process in very distinct ways. Mutans streptococci are believed to initiate the dental decay process within the enamel while lactobacilli are thought to be involved in the progression of decay further into tooth structure.[3,22]

Mutans Streptococci

Over the years, considerable epidemiological evidence has linked mutans streptococci to the development of caries and numerous laboratory investigations have demonstrated the ability of mutans streptococci to produce the lactic acid that cause dental decay.[21] Unfortunately for human dental health, mutans streptococci are a transmissible bacterial component of the plaque of most individuals. Mutans streptococci are gram-positive cocci that preferentially colonize the fissures on the occlusal and buccal surfaces of premolars/molars.[23] Additionally, they are acidogenic, enabling them to produce acids as a byproduct of their metabolism of dietary carbohydrates. The lactic acid they produce is responsible for the demineralization of tooth enamel as previously mentioned.[21] An additional characteristic that contributes to the increased cariogenicity of mutans streptococci is their aciduricity, bestowing upon them the capability of being able to survive in an acidic environment and maintain their acid production, even in a low pH environment.[21]

Carbohydrate metabolism is a key feature contributing to the virulence of mutans streptococci. Mutans streptococci are able to produce acid from carbohydrate metabolism due to an efficient glycolytic pathway, rapidly producing low terminal pH values in plaque.[23] The acid tolerance of mutans streptococci is based primarily on the presence of a membrane bound, acid stable, proton translocating ATPase that can maintain the bacterial intracellular pH at 7.5.[24] Therefore, mutans streptococci are able to survive and flourish in the presence of an acidic environment. Acidification of the local environment by the acid end-products of mutans streptococci inhibits the survival of

other competing bacterial species, enabling mutans streptococci to maintain their niche in the oral flora, incidentally causing dental caries in its host.[24]

Mutans streptococci are unique as they are able to metabolize a greater variety of carbohydrates than any other gram-positive organism sequenced so far.[24] Carbohydrates that are metabolizable by mutans streptococci include, but are not limited to: glucose, fructose, sucrose, lactose, galactose, mannose, cellobiose, melibiose, starch, and isomaltosaccharides.[24] This wide range of energy sources allow mutans streptococci a seemingly consistent fuel source for its survival.

Besides having an efficient process for metabolizing many carbohydrates, mutans streptococci have an efficient means of transporting sugar for their consumption. Mutans streptococci have high and low affinity sugar transport systems that are able to operate over a wide range of conditions, this ensures substrate uptake, even under extreme conditions such as low pH.[25] Therefore mutans streptococci are able to satisfy their metabolic demands even when the presence of fermentable carbohydrates is low.

Another factor that contributes to the high cariogenicity of mutans streptococci is their ability to store dietary sugars. Mutans streptococci produce intracellular polysaccharides (IPS) which store energy from fermentable carbohydrates when carbohydrates are present in abundance.[23,25] Storing sugars allows mutans streptococci to have a reserve energy source during conditions when there are no fermentable carbohydrates present in the host diet.[23,25,26] Additionally, mutans streptococci also produce extracellular polysaccharides (EPS) which act to consolidate the attachment of bacterial cells, possibly localizing acidic fermentation products, and reinforcing the

plaque matrix.[23] Together, IPS and EPS contribute to the establishment of an environment conducive to bacterial survival.

Mutans streptococci are further able to preferentially influence their bacterial colonization within the mouth by affecting the surface of the exposed tooth enamel. The bacterial cell surface of mutans streptococci contains ligands that act as receptors to bind to mucins (glycoproteins) that are found in saliva.[23] As the mucins become accumulated on the tooth surface, they begin to form a thin film that coats the tooth. This thin coating is known as an enamel pellicle. The enamel pellicle allows the cariogenic bacteria to closely bind to the tooth surface where they remain as they metabolize carbohydrates and produce destructive acid. Additionally, mutans streptococci produce the enzyme glycosyltransferase.[3,23] In the presence of fermentable carbohydrates, mutans streptococci are able to adhere through the polymerization of glucose derived from dietary sucrose into glucans.[23]

Although the general category "mutans streptococci" or MS is often found in dental literature it is important to mention that this is a term used to refer to a collection of bacterial species. *Streptococcus mutans* is considered by some to be the most cariogenic of all the mutans streptococcal species and according to Loesche the principle causative agent implicated in human dental caries.[24] *S. sobrinus* is the second most commonly isolated species of mutans streptococci in humans. Some studies indicated that this species might be more virulent than *S. mutans*. Clinical studies had found that subjects with a mixed infection of *S. mutans* and *S. sobrinus* tended to have more dental decay than subjects with infection of only a single mutans streptococci bacterial

species.[27] Other studies indicated that children with *S. sobrinus* infection developed more smooth surface decay than children without this mutans streptococci species.[27]

Acquisition of mutans streptococci is by transmission via saliva. Kohler and Bratthall[28] demonstrated that it was possible to transfer a sufficient amount of mutans streptococci by spoon from an infected parent to a non-infected child and establish a mutans streptococci infection in the previously uncolonized child.[28] As further support for the theory of mutans streptococci as a transmissible microorganism, Berkowitz and Jordan showed that children who became infected with mutans streptococccci possessed the same bacterial serotype as their infected mothers.[29] It is now well established that it is possible and probable that a parent or caregiver will transmit mutans streptococci to their previously uninfected child.[30]

Colonization of the oral cavity by *Streptococcus mutans* was proposed by Caufield and co-workers,[31] to occur during a specific window of infectivity. Caufield *et al.*, examined infants and found that *Streptococcus mutans* was commonly first isolated and detected within the oral flora between 19 and 31 months of age.[31] The idea of a "window of infectivity," an age range when mutans streptococci could be transferred to a previously uninfected individual and establish colonization, became mainstay in dental microbiology literature. Later studies by Mohan *et al.*, produced evidence in conflict to this commonly held belief. In 1998 Mohan and his colleagues demonstrated *Streptococcus mutans* could be detected in predentate infants.[32] Before this discovery, it was widely thought that the presence of a tooth surface, a non-shedding structure, was a necessary component in establishing *Streptococci mutans* infection. Tanner and co-workers showed high association between species detection from tooth and tongue

samples.[33] Together, these studies suggest that infants may be colonized by *Streptococci mutans* well before any teeth are present. These findings imply that intervention of oral bacteria transfer from caregiver to child should occur shortly after birth and may be fundamental in inhibiting the colonization of mutans streptococci in the mouth.

The presence of mutans streptococci on a tooth surface does not mean that caries will undoubtedly occur, only that the risk of caries development is increased. There have been many studies that have examined the relationship between the level of cariogenic bacteria in saliva and the estimated caries risk for particular individuals. Van Houte and Green determined that a critical level of 43,000 colony forming units of mutans streptococci per milliliter of saliva was necessary to establish a smooth surface infection.[34] Through research it has been determined that large concentrations of mutans streptococci seem to be necessary to establish infection in the fissures of premolars and molars, while even larger concentrations of this cariogenic bacteria are required to cause dental decay on the smooth surface of a tooth. Kohler and Bratthall concluded that caries-free children usually had less then 10,000 colony forming units of mutans streptococci per milliliter of saliva.[28] This suggests that while cariogenic bacteria may be present, suppressing their levels may allow individuals to maintain dental health.

There is great variability among individuals in levels of mutans streptococci present in the mouth. The reasons for some individuals having a greater concentration of mutans streptococci and being more "heavily loaded" are not clear. Individual levels of mutans streptococci and other antagonistic bacteria may be determined by the interactions of several factors such as diet, irregularities in enamel surfaces, presence of

increased retention sites, and heavy colonization of mutans streptococci in persons within close vicinity.

Lactobacilli

Various lactobacilli species have also been consistently associated with caries and are thought to be important secondary pathogens in the process of dental decay.[35] Lactobacilli are non-spore-forming, gram-positive, rod shaped cariogenic bacteria, they are highly acidogenic with the ability to produce lactic acid from the fermentation of glucose and other carbohydrates.[21,23]

Lactobacilli tend to colonize the mouth early in life and comprise a relatively small portion of the total oral microflora, usually less than 1% of the total cultivable microflora.[23] The establishment of lactobacilli is highly dependent on the presence of retention sites. Unlike mutans streptococci, which seemingly need teeth to serve as retention sites, lactobacilli may colonize the mouth in the absence of teeth. This is because lactobacilli are able to use oral surfaces such as the tongue and cheek for colonization.

Lactobacilli have been associated with the presence of dental decay and are thought to be more active within carious dentin and at the advancing front of caries lesions rather than in the initiation of the carious lesion. Additionally, the prevalence of lactobacilli may increase as dental caries progress or as the patient's dietary carbohydrate intake increases.[23] High counts of lactobacilli in saliva and plaque are often indicative of a high total carbohydrate consumption and is therefore considered an indirect indicator of caries risk.[35]

Although lactobacilli counts have been used to predict increments of new cavities, these counts alone are not considered reliable enough to predict dental caries and are a more reliable indicator of increased carbohydrate intake.[23] While bacterial counts can be used as an adjunct for helping to determine a patients caries risk, they should be used as a part of a more extensive caries risk determination program.

1.4 Host Factors

Many host factors can have a direct effect on the balance of protective and pathogenic factors. Factors that can change this balance and possibly increase a patient's caries risk include the mechanical and chemical functions of saliva, irregularities in the enamel surfaces of the tooth, and tooth position. All of these factors including genetic and immunologic considerations can play a role causing disturbances in protective factors and increasing pathogenic factors favoring caries initiation/progression.

Saliva

The host defense systems against oral pathologic factors are primarily found in the saliva; therefore an adequate salivary flow is necessary to maintain dental health. The benefits of saliva are extensive, as it serves a multitude of functions. First of all, saliva is important for the mechanical clearing away of foods and bacteria from the tooth surface.[23,26,36] Additionally, saliva contains bicarbonate that help to neutralize the acid byproducts from bacterial carbohydrate fermentation as well as neutralize acidic food sources. Therefore saliva helps to maintain a relatively constant pH in the mouth. Saliva also serves as a reservoir for calcium, phosphate, fluoride, and other minerals necessary

for remineralization of enamel.[21,36] Saliva also contains many antimicrobial proteins and organic compounds such as lysozymes, peroxidase, and immunoglobulins, including antibodies against specific bacteria.[23,26] For all of these reasons, an adequate rate of salivary flow is needed to maintain the integrity of the tooth structure.

Tooth

Individual tooth characteristics may also contribute to a patient's susceptibility to developing caries. Lack of enamel maturation and the presence of developmental structural defects may increase the caries risk of an individual.[23,26] Irregularities in the tooth surface such as pits and fissures, particularly in posterior teeth, create additional retentive sites for bacteria to adhere to. Tooth irregularities not only provide a more favorable area for bacterial colonization, they also create areas not easily cleansable by salivary flow.[37] The compounded effect of tooth irregularities support bacterial establishment and increase the patients caries risk. Additionally, lack of enamel maturation may serve as a predisposing factor to high caries risk. Reduced enamel quantity and quality significantly decreases the resistance of teeth to even a relatively mild acid challenge.[26]

Another important factor that may influence caries risk is tooth position. Positional anomalies and deviations, including crowding and overlapping smooth surfaces, make oral hygiene a greater challenge for an individual to accomplish successfully. Because deviations in position often allow for more contact area between teeth, it is more difficult to mechanically remove plaque, unlikely that saliva will be able to cleanse bacteria away, and there is less chance of exposing overlapping tooth surfaces

to beneficial effects of fluoride. All of these tooth factors increase the caries risk the individual is likely to experience.

1.5 Diet

Another important host factor is the dietary profile of the patient. A change in the quantity of carbohydrate consumed and the frequency of carbohydrate consumption can affect the balance between protective and pathologic factors. Even in the presence of cariogenic bacteria, if little or no suitable dietary substrates are present, little or no acid will be produced through carbohydrate metabolism, consequently the risk of caries development will be low. Dietary habits can also influence the composition of the oral flora.

Frequency of consumption of fermentable carbohydrates can also influence caries challenge. Increased frequency of carbohydrate consumption (i.e. sucrose, glucose, fructose, cooked starch) increases the acidity of plaque and prolongs the length of time that an acidic environment is maintained in the mouth. The extended time that an acidic environment is maintained by the presence of fermentable carbohydrates, increases the potential for enamel demineralization, and provides inadequate time for remineralization of dissolved tooth structure by various components of saliva. The result is there is an imbalance between the protective and pathological factors in which the pathological factors that favor demineralization becomes the dominant mechanism.[21]

1.6 Treatment of Caries

Restorations

Until recently, clinical management of dental decay consisted solely in physical removal of decay from the tooth and restoration of the defect with a stable material. Dental restorations are commonly placed as a treatment of dental decay. It has been shown that physically excising the diseased portion of the tooth with its bacteria components does not result in any measurable long-term reduction of cariogenic bacteria loading.[15,38] Gregory *et al.*, showed that there were no significant differences in pre- and post-restoration concentrations of mutans streptococci or total oral streptococci in children.[38] It has been shown that removal of decay and placement of restorations does have a temporary effect of decreasing level of mutans streptococci and lactobacilli, but shortly thereafter these levels gradually returned to the pre-treatment levels in most subjects studied,[15] unless chlorhexidine antibacterial therapy was used.[17] Therefore, standard restorative treatment alone is not effective for eliminating mutans streptococci or lactobacilli from tooth surfaces throughout the mouth or eliminating caries risk. Considering that dental restorations are the currently accepted protocol treatment for dental decay, and that this treatment has no significant effect on the patient's risk for future development of caries, it is imperative that alternate treatment options be explored.

These combined observations support the idea that even if cariogenic bacteria are mechanically removed from the accessible tooth surfaces, these bacteria are able to re-establish themselves over time, presumably from reservoirs that are not accessible. Because of the demonstrated ability of cariogenic organisms to rapidly re-establish throughout the mouth it is essential to effectively reduce the numbers of salivary

cariogenic bacteria in the plaque by antibacterial treatment in order to have a significant effect on an individual's caries risk.[38]

1.7 Prevention of Caries

Caries has been viewed as a bacterially mediated disease since the late 1800's. In 1976 Dr. Walter Loeshe developed the Specific Plaque Hypothesis which postulated that there were a finite amount of organisms in dental plaque that were responsible for causing the caries process.[39] Since that time, dental research has validated that dental caries is a bacterial infection and that numerous species are involved. Unfortunately the exact mediators of disease have not been identified. It appears that many strains of bacteria contribute to creating an environment that supports the initiation and progression of dental decay.

Because caries involves many bacterial species, it is likely that a vaccine directed against one particular species will not necessarily have a beneficial effect on the overall caries status of the individual.[21] Therefore, it is believed that an antibacterial therapy is needed to reduce the bacterial challenge and allow the protective factors of the mouth to favorably shift the caries balance to support dental health.[21]

Restorative work is needed as a result of dental decay can be quite expensive, especially when the patient is young and has extensive decay or in the case that the patient is medically compromised. For this population it may be necessary to render restorative dental treatment under general anesthesia. Despite efforts in restorative therapy, children who experience early childhood caries continue to be at a higher risk for new lesions in both the primary and the permanent dentition. Interventions which disrupt

the pathobiology of caries are needed to prevent and treat this aggressive infectious disease.[40] In order to develop these strategies however, it is important that all bacteria associated with dental caries and dental health be identified.[41]

Although the concept of using antibacterial therapy is logical, as it is utilized in hospitals to treat bacterial infections, it is not widely used in the prevention of dental decay.[21] Tradition has dictated non-conservative restorative treatment. To date, there are no specific antibiotics or vaccines available to work against the numerous cariogenic bacteria that collectively cause dental decay.

Oral Hygiene

A study conducted by Wikner showed that oral hygiene measures taken by the patient, prior to sampling the saliva, may mask the true concentration of salivary mutans streptococci and complicate the identification of high caries risk patients.[42] Although Wikner's study supported the benefit obtained from the mechanical removal of plaque from teeth[42], it is general knowledge that toothbrushing alone is not sufficient to inhibit dental decay in the presence of high salivary concentrations of cariogenic bacteria.

Sealants

Traditionally, caries prevention has failed to address the prominent role of cariogenic microflora in the development of caries. Instead, prevention has focused on eliminating plaque from tooth surfaces, believing that by removing plaque the cariogenic bacteria were also removed. Dental sealants are now standard of care, allowing for deep pits and fissures which bacteria can use as retention sites to be covered over. The

emerging philosophy of individualized/comprehensive caries control involves preventing further development and progression of the disease process and reversing the process where possible.[43]

Fluoride

Fluoride, which has been utilized in oral health since the 1940's, is known for its well-established effects on the remineralization processes.[21] It is an anion that when incorporated into the apatite of the tooth structure can decrease tooth solubility making it more resistant to acid challenge.[5] It is available for delivery to individuals in many forms including, but not limited to: liquid vitamins, tablets, toothpastes, mouthrinses, water, chewing gums, and varnishes.

Fluoride can also act as an inhibitor of cariogenic bacteria.[44] Fluoride can not enter the cell as the ion, but can readily diffuse into the cell as HF, so when the bacteria produce acid, the hydrogen ion combines with fluoride present in the plaque and diffuses into the cells.[5] Inside the cell the HF dissociates, producing fluoride ion when interferes with enzyme pathways. Fluoride interferes with enolase, a critical enzyme in the metabolic pathway, thereby reducing the acid by-product.[5] Additionally, fluoride may act to reduce the function of the membrane gradient for H^+ which directly inhibits the glucose uptake necessary for bacterial metabolism to occur at low pH.[5,44]

The duration of the effect that fluoride exercises within the mouth depends upon its concentration and the vehicle used for its delivery. Critical levels of fluoride are need for its beneficial effect. The remineralization of demineralized enamel requires less than 100 μg/ml of NaF, while the inhibitory effect of fluoride upon bacteria requires a

concentration of greater than 100 μg/ml of NaF. In order for fluoride to exert bacteriocidal effects there needs to be a 30 fold increase in its normal oral concentration.[45]

Fluoride is routinely used to reinforce good oral hygiene, but its use has been shown to be effective only up to a point; a high bacterial challenge cannot be completely overcome by even high-concentration fluoride therapy.[21]

Sugar Alcohols

Sugar alcohols are artificial sweeteners that have recently been incorporated into commonly consumed products. Sugar alcohols are considered non-cariogenic since they have the ability to reduce or even prevent drops in pH under adverse conditions.[23] It has been shown that when xylitol, a popular new sugar alcohol, is incorporated into chewing gum and used consistently over a period of three months, it appears to have the ability to prolong the effect of chlorhexidine therapy on reducing oral mutans streptococci.[43] Sugar alcohols are becoming more popular as more products containing this sugar substitute are being developed. Currently, some available products that contain sugar alcohols include chewing gums, lozenges, dentifrices, and saliva substitutes.

Sugar alcohols usually consist of a combination of hexitols and pentinols. In 2003, Lynch and Milgrom reviewed xylitol and concluded that it had the ability to inhibit the establishment of cariogenic bacteria and significantly reduce the incidence of caries.[46] It is speculated that this reduction may be attributed to numerous mechanisms. Sugar alcohols may replace sucrose, thereby reducing the availability of fermentable carbohydrates available to cariogenic bacteria for their metabolism.[23] This directly

decreases the frequency of acid production. Products such as chewing gums and lozenges which normally increase salivary flow (with its protective factors), the addition of sugar alcohols have the overall effect of encouraging remineralization by creating an environment where saliva can exert its mechanical and chemical effects while sugar alcohol helps to keep the acidity of the mouth near neutral.[23]

In the case of xylitol, its specific metabolic action is understood. When xylitol is ingested by bacteria, the microorganism attempts to get rid of the non-metabolizable product, requiring energy, thereby creating a negative energy cycle in which the bacteria looses energy without producing any acid byproduct.[23] As this process tends to reduce both the growth rate and the acid production, it can possibly lead to reduced levels of mutans streptococci and dental decay in habitual users of xylitol containing products.[23]

Substitution of sugar alcohols for sucrose is highly recommended for patients who suffer from hyposalivation or who have a high caries risk, as sugar alcohols decrease or eliminate acid production.

Unfortunately, sugar alcohols (like xylitol or sorbitol) can have adverse laxative effects. It has been shown that ingesting greater than 15 grams of sorbitol may cause diarrhea as the result of osmotic effects in the intestine and the fact that sugar alcohols are absorbed slowly.[45]

1.8 Antibiotic Treatment

Non-ionic Antiseptic Agents

Non-ionic agents such as Listerine have been utilized for decades as skin antiseptics. Listerine is the brand name of a product that contains phenol derivatives and

essential oils. Non-ionic derivatives exhibit both hydrophilic and hydrophobic properties. These characteristics confer upon these substances a broad spectrum of activity enabling them to exert their effects upon a wide variety of oral gram-positive and gram-negative bacteria.[45] How well non-ionic agents work is determined by the concentration of the agent and its substantivity.

While the mode of action of non-ionic agents is not entirely understood, it has been suggested they exert antibacterial effects. They are able to reduce the uptake of glucose, which results in decreased acid production. Additionally, in high concentrations, these agents causes bacterial membrane leakage which leads to bacteriolysis.[45] In a study conducted by Fine *et al.*, it was found that the essential oil mouthrinse (Listerine) produced respective reductions of 69.9% and 75.4% in total recoverable streptococci and in mutans streptococci in plaque.[47] There were also corresponding reductions of 50.8% and 39.2% in saliva.[47] However, there are no studies showing that these reductions are sustained or that there is an effect in reducing dental caries.

Listerine has a strong taste and a high concentration of alcohol.[45] Because of Listerine's high alcohol concentration, it is not recommended for recovering alcoholics or young children.

Chlorhexidine

The effects of chlorhexidine were first described by Davies *et al.*, in 1954.[45] Chlorhexidine was used as a dental plaque inhibiting substance by Loe and Schiott in 1970.[45] Chlorhexidine is broad spectrum antimicrobial agent that can be directed against

yeasts, fungi, and a wide range of gram-positive and gram-negative bacteria.[23] It is commonly utilized in dentistry to reduce high levels of mutans streptococci and periodontopathogens, but has shown to be ineffective against lactobacilli.[48,49] Patients considered to have high periodontal disease activity can be treated with a chlorhexidine regimen as well as patients who exhibit high counts of mutans streptococci or have high caries activity.

Vehicles available for delivery of chlorhexidine include: solutions, gels, and varnishes.[23] The varying efficacy of chlorhexidine is dependant upon the vehicle of delivery, concentration, substantivity, duration of treatment and cooperation of patient.[45]

Chemically, chlorhexidine consists of 1,6-bis-p-chlorophenylbiguanidohexane.[45] Chlorhexidine is a positively charged cation that is attracted to the negative charge of bacterial cell walls.[49] Gram-positive bacteria such as mutans streptococci, have a more negatively charged cell wall and are thus more sensitive to cationic substances such as chlorhexidine.[45] Chlorhexidine binds readily to negatively-charged bacterial cell walls and disrupts the integrity of the bacterial membrane.[49] When chlorhexidine is in the presence of fluoride, fluoride has an antagonistic effect. When fluoride interacts with chlorhexidine, the small negatively charged fluoride ion inhibits the bactericidal effects of chlorhexidine on cariogenic bacteria. At higher concentrations, chlorhexidine is bactericidal and acts as a detergent by directly damaging the cell membrane.[23]

Another distinctive property of chlorhexidine is its ability to bind to oral surfaces from where it is released gradually into saliva over many hours at bacteriostatic concentrations, allowing for a chlorhexidine reservoir.[23] Commonly reported undesirable effects arising from the use of chlorhexidine include discoloration of teeth, tongue,

restorations, and dentures.[23,49] Additionally, there have been reports of soreness of the oral mucosa and temporary taste disturbances. These untoward effects are generally related to the 0.2% chlorhexidine mouthrinse.[45]

Povidone Iodine

In the early 1950's, povidone iodine was determined to be a powerful broad spectrum antibacterial that demonstrated effectiveness against a wide variety of bacteria, viruses, fungi, protozoa, and spores.[50] Clinically, povidone iodine has been employed in the management of wounds, as a pre-surgical skin antiseptic, and as a disinfectant. Previous studies by Newacheck *et al.*, and van Houte *et al.*, have indicated that povidone iodine is potentially lethal to mutans streptococci.[1,35] Recent studies in children have indicated that topical antimicrobial therapy with povidone iodine every two months may reduce caries and significantly increase the proportion of disease-free children at high risk for developing early childhood caries.[51] While the Federal Drug Administration has approved solutions of 10% povidone iodine safe for the application to mucosa, povidone iodine is not currently utilized in routine dental rehabilitation, and its related efficacy in decreasing the risk for dental decay is unknown.[52]

Iodine is an element that appears naturally as a dark violet solid. It has previously been used as a hospital antiseptic that has been known to cause sensitivity, irritation, and burning when used in its pure form. Recently, there has been some interest about its effect on cariogenic bacteria. As reviewed by Den Besten and Berkowitz, it is possible that iodine therapy may be more effective than chlorhexidine against cariogenic bacteria.[14]

Povidone iodine is available commercially as a Betadine, a 10 percent povidone iodine solution. A 10% povidone iodine solution contains 90% water, 8.5% polyvinylpyrrolidone and only 1% of available iodine and iodide.[53] It is considered safe and has long been used in general clinical practice as a pre-surgical antiseptic.

Polyvinylpyrrolidone povidone iodine (PVPI) is an iodophor consisting of a combination of a complex of iodine (I) with polyvinylpyrrolidone (PVP) surfactant.[54] Combining iodine with PVP increases its ability to dissove in water, reduces its irritability and decreases the staining caused by pure iodine. When found in its PVPI bound form, iodine is inactive and asserts no effect.[54] Due to the fact that PVP is a water solubilizing agent, in the presence of water, PVP liberates a small amount of free iodine to solution.[54] When iodine dissociates from the PVP complex, it becomes biologically active. When iodine is in its unbound form, it is active and able to assert its germicidal action.[55] The equilibrium between the complex-bound iodine and the non-complex bound iodine is maintained as the active non-complex bound iodine is used up in killing microorganisms it is replaced by iodine from the complexed form. This will continue until the PVPI reservoir has been exhausted.[55]

While the exact mechanism of action for povidone iodine is unknown, there are many studies that suggest that it exerts its effects on multiple cellular levels. Povidone iodine has been shown to have effects on important cellular structures including proteins, nucleotides, and fatty acids. It is believed that povidone iodine's main effect results from unbound iodine acting on the respiratory enzymes found in the membranes of microorganisms.[55] Povidone iodine is thought to be able to oxidize the S-H bonds of cysteine and methionine, react with the phenolic groups of tyrosine, and react with N-H

groups in amino acids to block hydrogen bonding. Additionally, povidone iodine reacts with bases of nucleotides to prevent hydrogen bonding and alters membrane structure by reacting with C=C bonds in fatty acids.[56] Amino acids are essential to make new proteins within a cell, if these are negatively altered by iodine, the cell will be unable to produce the proteins it needs to survive and the cell will eventually die.[55] Therefore, if active iodine comes into contact with the bacterial cell membrane, the cells functions will become disrupted and the microorganism will die.[55]

Allergic reactions to povidone iodine are rare, occurring in the general population at a rate of one person in one-thousand.[55] Most of the allergic reactions previously associated with iodine, including irritation, sensitivity, skins burns, bad taste, and staining have been eliminated with the development of iodophores in the late 1940's.[56] Iodine toxicity symptoms include fever, diarrhea, mental status alteration, metabolic acidosis, abnormal thyroid function, hypernatremia, neutropenia and hepatic failure.[54]

There is some controversy as to povidine iodine's overall biological effect. While animal models have indicated that PVPI exhibits cytotoxicity against crucial body cells, including leukocytes, fibroblasts and keratinoctyes,[54] human studies on PVPI's effect support the idea that PVPI reduces bacterial load, decreases infection rates and promotes the process of healing.[56]

2. SIGNIFICANCE OF THE STUDY

Dental decay, a transmissible bacterial infection, is the result of acid produced by bacteria. In the caries process, cariogenic bacteria (mutans streptococci and lactobacilli) in dental "plaque" on the tooth surface feed on dietary carbohydrates and produce acid, which dissolves calcium and phosphate from the tooth mineral in a sequence known as demineralization. As demineralization progresses, it eventually produces a cavitation in the tooth structure which requires restoration in order to eliminate infection and pain. If the oral environment is manipulated so that protective factors (salivary components and antibacterials) dominate over pathologic factors (MS, LB, carbohydrate intake, decreased saliva) then reincorporation of minerals into the demineralized tooth surface is possible. Individual fluctuations in the balance between these factors determine whether dental decay will occur.

The incorporation of fluoride has aided in the decline of overall caries experience since the mid-1970's, but its beneficial effects appear to have reached a plateau. While excellent oral hygiene (brushing and flossing) is advocated as it removes plaque, it has been shown that the mechanical removal of plaque from the tooth does not have any long-term effect on bacterial levels in the mouth. Despite these methodologies and procedures currently in use, a portion of the population continues to remain at considerable risk of caries initiation and progression. These high-risk individuals are most likely in need of added preventive measures such as antibacterial treatment to reduce their caries challenge.

Numerous studies have shown mutans streptococci (MS, which includes *S. mutans* and *S. sobrinus*), and lactobacilli (LB) to be the major cariogenic bacteria in

adults and children.[21,57-59] Although the concept of using antibacterial therapy is logical, as it is utilized in medicine to treat bacterial infections, it is not widely used in the prevention of dental decay.[21] Because there are numerous species and strains involved in the caries process, broad range antibacterials are probably the best choice to deal with dental caries.

Currently, chlorhexidine is the most often used antibacterial for caries prevention, but it has yielded limited success. Studies show chlorhexidine products reduce the levels of *S. mutans* in the mouth, but its efficacy on lactobacilli is limited.[60-63] A disadvantage of chlorhexidine use continues to be the difficulty of maintaining patient compliance. Not only does it taste unpleasant, it may also cause temporary tooth discoloration. The chlorhexidine treatment regimen includes daily rinses for at least two weeks every three months, or one week every month to reduce the MS loading in the mouth by about 60%. Elimination of cariogenic bacteria using chlorhexidine is very difficult and other antibacterials such as cetyl pyridinium chloride are ineffective. Therefore, an antibacterial with increased efficacy on cariogenic bacteria with improved patient acceptance is needed.

Recent studies in children have indicated that povidone iodine used every two months may reduce caries.[64] Additionally, previous studies indicated its potential use in killing *S. mutans*.[65,66] Although it is not yet known whether povidone iodine will be more effective than chlorhexidine in killing *S. mutans*, *S. sobrinus*, or lactobacilli, the present study addressed this important possibility.

In order to improve the taste of povidone iodine and improve its ease of clinical administration, a 10% fruit flavored povidone iodine foam was used in the present study.

The efficacy of povidone iodine against mutans streptococci and lactobacilli and its effective duration following a single application on children with high caries risk was investigated.

Povidone iodine foam was expected to have the same efficacy as the previously used povidone iodine solution. The advantage expected by using this antibacterial in a foam vehicle was improved taste and easier application for routine treatment.

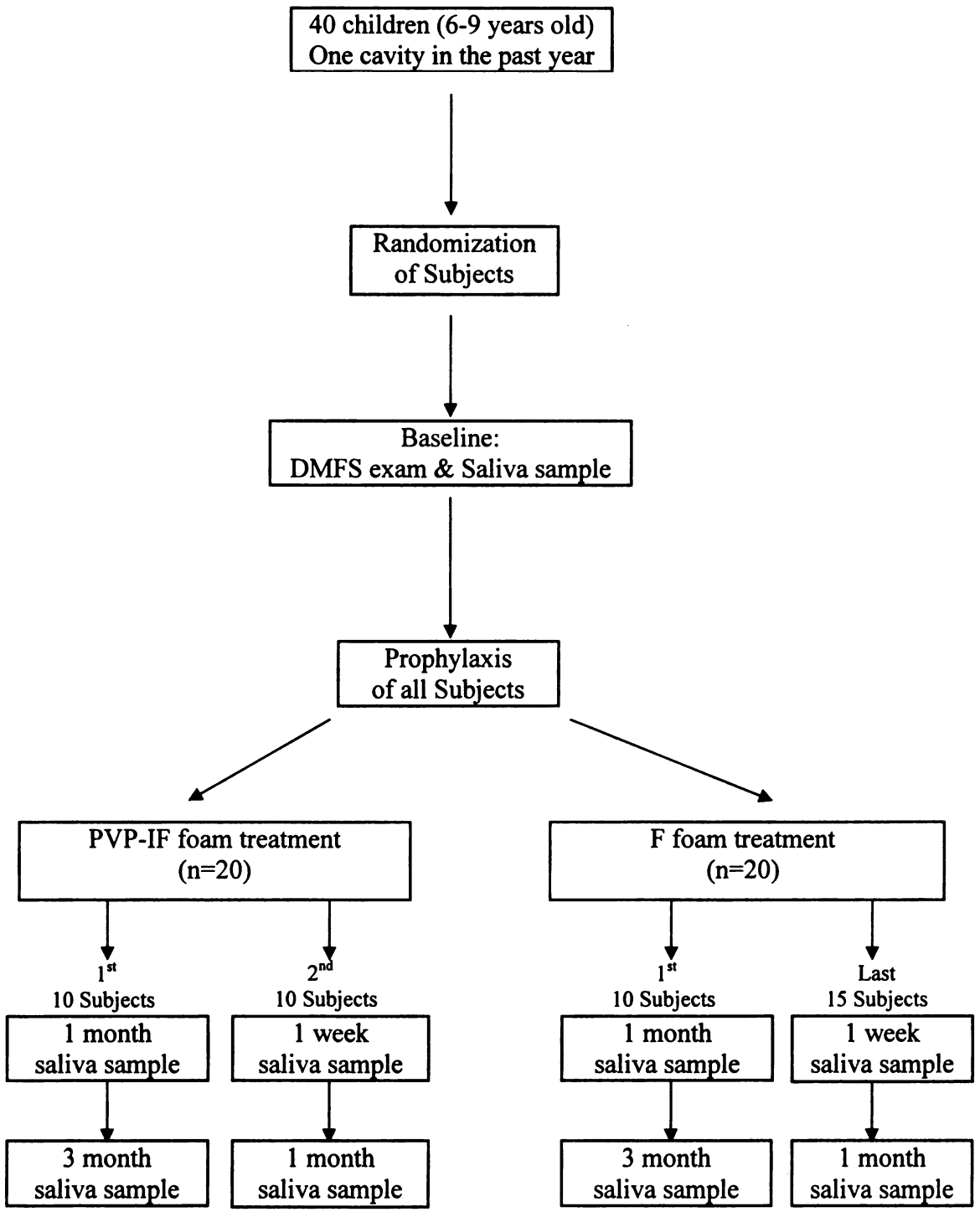
3. SPECIFIC AIMS

The aim of the study was to determine the clinical antimicrobial efficacy of a single-dose treatment of povidone iodine foam against cariogenic bacteria, namely, *Streptococcus mutans*, *Streptoccocus sobrinus* and *Lactobacillus* species in children with active caries. Additionally, this study allowed us to compare the effect of povidone iodine treatment with standard fluoride treatment and evaluate the patient acceptance of the taste of the prototype povidone iodine foam.

4. MATERIALS AND METHODS

4.1 General Study Design

Figure 1 Study Design Flow Chart



4.2 Subject Recruitment, Randomization, and Blinding

The UCSF Committee on Human Research (CHR) approval (#H8693-22998-01A) was obtained prior to recruitment of any subjects. Before any exam, sampling, or treatment was rendered, parents or legal guardians of eligible subjects/children, received a verbal and written explanation of the purpose of the study as well as the outlined study protocol. A signed informed consent, previously approved by the UCSF CHR, was obtained from the guardian/parent who agreed to have their child participate in the study. The nature of the study, the details of what was involved, and any questions pertaining to the study were answered. Children who agreed to participate in the study signed an informed assent, previously approved by the UCSF CHR.

Aside from the collection of saliva samples, all dental procedures were similar to what each subject would otherwise normally have received during a periodic oral exam. There was no extra cost accrued to the parents/guardians for their child's participation in this study.

The study population consisted of forty children who were patients in either the Pediatric Dental Clinic in the Division of Pediatric Dentistry at UCSF, or at the Tenderloin Community School Dental Clinic, a UCSF satellite office. There were 17 males and 23 females who ranged from ages 6-9 years old at the start of the study. Subjects were selected based on appropriate age range and history of past (within 1 year) or present dental decay. At UCSF subjects were recruited at the time of their scheduled routine dental exam and at the Tenderloin Dental Clinic, recruitment was based on parents interest of their child's participation in the study.

Inclusion criteria for subjects enrolled in the study included:

- Patients with a dental history of at least 1 active dental cavity within the last year.
- Residents in San Francisco or within a 20 mile radius of San Francisco.
- Parent and child both willing to participate in the study
- Parent/guardian who can speak and read English, Spanish, or Chinese.

Exclusion criteria for subjects interested in study participation included:

- Children with serious chronic system diseases
- Children with a medical history which included antibiotics taken within the past 3 months
- Children with periodontal disease that required regular periodontal treatment
- Children with a dry mouth or who had difficulty spitting

A randomization sheet was used to assign patients to either the control or intervention group. The clinician administering treatments assigned each subject either treatment foam A or treatment foam B based on the randomization sheet. The clinician and the personnel involved in microbiology analysis only knew of the subject's assignment to group A or B, but were blinded to which group of subjects were assigned to the "control" or "intervention" group.

4.3 Examination

The subject's medical history was reviewed and either the presence of at least one carious lesion was clinically confirmed, or the subject's history of having at least one dental carious lesion in the past 12 months was confirmed. Charting was completed with

the use of a dental mirror and an explorer under a routine pediatric dental clinic setting. The exam was supplemented with selected periapical and bitewing films as indicated. The following information was included on a modified dental charting sheet for each subject: teeth present, carious surfaces, restored surfaces and teeth missing as a result of decay. This index was updated for each subject at each visit.

Teeth with explorer detectable cavitations and teeth with radiographically observable interproximal radiolucencies (not associated with normal tooth anatomy or enamel hypoplasia) were deemed valid carious lesions. If a non-cavitated white spot lesion was observed on a tooth, it was not included, as it was not considered a carious lesion by our study standards. For reasons of validity and continuity of patient care, the same clinician (NB) was responsible for performing all exams, radiographic interpretation, dental charting, saliva collection, prophylaxis, and administration of treatment.

The information obtained from the intraoral examination and radiographic examination (when appropriate) was used to compile a dmft/DMFT (decayed-missing-filled teeth) and dmfs/DMFS (decayed-missing-filled surfaces). Lowercase letters were used to specify primary dentition and upper case letters were used to specify secondary dentition. Missing teeth and surfaces were included in documentation only when the dental chart indicated a history of extraction due to dental decay.

4.4 Traditional Stimulated Saliva Collection Technique

After examination, a sample of the patient's saliva was collected via the traditional stimulated saliva collection technique. Stimulated saliva was used to give an

overall sample of the whole mouth loading of MS, LB and Total flora in the plaque. Saliva flow was stimulated by having the subject chew on a 2x2 cm piece of paraffin (Parafilm "M" Laboratory Film, Pechiney Plastic Packaging, Chicago, IL) for 1 minute, followed by expectoration into a 5 ml sterile vile (Falcon, USA). One ml of stimulated saliva was collected from each subject for microbiological assessment prior to any dental treatment and the sample was stored in the refrigerator immediately after collection, and processed within 24 hours.

4.5 Prophylaxis Technique

After the saliva collection at the initial visit, a thorough prophylaxis using a disposable prophylaxis cup (Denticator, Earth City, MO) and medium grit prophylaxis paste (Sultan Dental Products, Englewood, NJ) was performed. All teeth were flossed and rinsed with water. Excess water was removed from the mouth with low vacuum suction.

4.6 Treatment Administration

To assist in both the blinding of the clinician and patient to random group assignment and to increase the patient acceptance of the PVPI-F foam, Omnii Oral Pharmaceuticals formulated a PVPI-F and a F solution which were administered from identical clear foam dispensers. A reddish brown tint was added to the F solution to blind the clinician and give a similar appearance to both solutions and a blueberry flavoring was incorporated into both solutions to aid in masking the bitter taste of PVP-IF. Upon receiving the specially formulated foams, the study's principal investigator labeled the PVPI-F foam "Foam A" and the F foam "Foam B."

After prophylaxis, either foam "A" or "B" (based on the patient's randomization assignment) was placed into disposable foam fluoride trays (Funtrays size small and medium, Oral B laboratories, Redwood City, CA). The foam trays with treatment foam were placed into the patient's mouth for 4 minutes with a low volume vacuum to remove foam that overflowed from the trays into the patient's mouth. Upon completion of treatment, trays were removed and the patient was allowed to expectorate in the sink. Patients were instructed not to drink anything, including water, nor eat anything for 30 minutes. The patient acceptance of the foam, staining of the teeth, and any other complaint were reviewed immediately after the foam treatment. Possible long-term side effects and complaints such as allergy, stain, or oral irritation were also reviewed and recorded at each follow-up visit.

4.7 Saliva Collection Recalls

The original study design set patient recalls at one month and three months after treatment. Halfway through the study, based on a lack of significant changes in bacterial levels from one to three month time intervals, and believing that this time interval was beyond our window of interest, the recall times were adjusted and patients were followed-up one week and one month after treatment. Therefore, the distribution of recalls was as follows: all subject in both groups returned for one-month follow-up visits, the first 10 patients from each group returned three-months after baseline, and the second 10 patients in the intervention group and the following 15 subjects in the control group were called for a one-week follow-up visit.

3.8 Laboratory and Microbiology Procedures

The acquired saliva sample was placed in a refrigerator at 37°C until laboratory transport. Samples were transported to the UCSF laboratory within 5 hours of collection in a Styrofoam transport container with a cold pack placed inside. The tubes were uniquely coded for each subject and visit number. The microbiologist and other laboratory personnel responsible for plating saliva samples and quantifying bacterial content were blinded to subject assignment to control/intervention group. All saliva samples were plated for mutans streptococci (MS), lactobacilli (LB), and total viable count (TVC) within 24 hours of collection.

Each saliva sample was sonicated for 20 seconds to disaggregate and evenly disperse bacterial cells. Each sample was serially diluted in phosphate buffered saline (PBS) from 10^{-2} to 10^{-6}.

One-tenth of a milliliter of undiluted saliva and one-tenth of a milliliter of each dilution was placed on Mitis Salivarius Sucrose Bacitricin (MSSB) agar for mutans streptococci enumeration, on Tomato Juice-Rogosa Agar for lactobacilli enumeration, and on brain heart infusion agar containing 5% sheep blood for total viable count of bacteria.

All plates were incubated in 85% nitrogen, 10% hydrogen and 5% carbon dioxide at 37°C. Plates were incubated for 48 hours before enumeration. Bacterial counts were expressed as $\log_{10}$ CFU/ml of saliva.

3.9 Statistical Analysis

The colonization levels of the targeted bacteria were determined for all subjects at each visit. The obtained values were non-parametric and were therefore converted to $\log_{10}$ values so that parametric statistical tests could be applied to the collected data. Means and standard deviations in log MS, log LB, and log TVC were calculated for each group at every time interval. For MS and LB, percentages of raw bacterial counts were also calculated against TVC as a parameter to measure bacteria loading.

Baseline comparisons of the two groups were performed to establish validity of the randomization process. Student t-tests were performed to determine any significant differences in the log values of mutans streptococci and lactobacilli at one-week, one-month, and three-months from baseline in both the PVP-IF and F groups. The results were used to compare the short term and long-term impact of PVP-IF and F treatment on MS, LB or TVC, specifically the fluctuation in bacterial loading and the length of time any notable effects lasted.

5. RESULTS

5.1 Subjects at Baseline

A total of 40 children (17 males and 23 females) were enrolled in the study with 20 subjects in each group. At baseline, the mean age of the subjects was 7.20 years (range: 6-9) in the intervention (PVPI-F) group and 7.45 years (range: 6-9) in the control (F) group (Table 1). There was no statistically significant difference in gender distribution and age between the two groups ($P>0.05$).

Table 1 Subject Demographic Parameters

	Intervention Group	*Control Group*
Treatment Type	PVPI-F Foam	F Foam
Average Age	7.20 years	7.45 years
Male: Female	10:10	7:13

Table 2 shows the mean dmfs/DMFS and the mean levels of mutans streptococci, lactobacilli, and the total viable count calculated for each group at baseline. All subjects enrolled in the study were confirmed to have at have had active dental decay within the past year. There were only three subjects in the PVPI-F group and one subject in the F group who did not have any detectable active dental decay at baseline. The mean dmfs/DMFS was 12.15 (range: 2-32) in the PVPI-F group and 15.27 (range 1-46) in the F group. The mean ds/DS was 3.85±4.13 (range: 0-19) in the PVP-IF group and 6.15±7.40 (range:0-31) in the F group. There was no statistically significant difference in dmfs/DMFS and ds/DS between the two groups (Student t-test, $P>0.05$).

When considering microbiology, all but one subject in both groups had high ($>10^5$ CFU/ml) MS levels. Fourteen (70%) subjects in the PVPI-F group and thirteen (65%) subjects in the F group had LB infection. The TVC in both groups was comparable between the log values of 7-9. There were no statistically significant differences found in log MS, log LB, and log TVC between the two groups at baseline (Student t-test, $P>0.05$).

Table 2 Mean (SD)* $\log_{10}$ Bacterial Counts at Baseline

	PVP-IF	*F*
dmfs/DMFS	12.15 (9.14)	15.27 (12.36)
Log_{10} MS	4.87 (1.37)	4.79 (1.54)
Log_{10} LB	2.79 (2.24)	2.77 (2.49)
Log_{10} TVC	8.35 (0.50)	8.26 (0.56)

*denotes standard deviation of the mean

5.2 Subjects at Completion

Numbers of subjects from each group who returned at each visit are noted in Table 3. In the PVPI-F group, one subject missed the three-month follow-up visit, the remaining subjects in this group completed all follow-up visits. In the F group, one subject missed the one-month follow-up visit and one subject missed the three-month follow-up visit, all other subjects in this group completed all follow-up visits.

Three subjects in the PVPI-F group complained of an unpleasant taste of the PVPI-F foam, while two subjects in the F group complained of an unpleasant taste of the F foam. All of the 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 resulting from foam treatment were noted in either group.

The restored ds/DS was calculated for every subject at each visit. No subjects in either group received restorations at one-week. However, the F group had more restorations placed and more subjects who received dental restorations at one-month and three-months when compared to the PVPI-F group. The differences were statistically significant at one-month after baseline for restored ds/DS (t-test, P=0.041) and for the

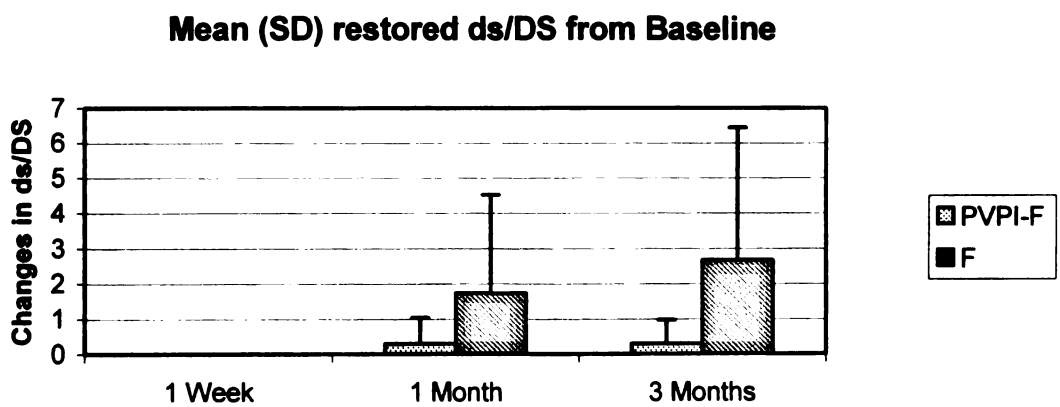
percent of subjects between the two groups who received restorations (Pearson Chi-Square test, P=0.022). Table 3 demonstrates the mean restored decayed surfaces in both the PVPI-F and F groups, as well as the percentage of subjects in each group who received restorations at each follow up visit.

Table 3 Mean (SD)* Cumulative Restored ds/DS at Various Time Intervals

Time	*PVPI-F*			*F*		
	n	Mean (SD) restored ds/DS	% of subjects with restorations	n	Mean (SD) restored ds/DS	% subjects with restorations
1 week	10	0.00 (0.00)	0	15	0.00 (0.00)	0
1 month	20	0.30 (0.73)	20	19	1.74 (2.79)	58
3 months	9	0.30 (0.67)	25	8	2.67 (3.77)	63

*denotes standard deviation of the mean

Figure 2 Mean (SD) Cumulative Restored ds/DS at Various Time Intervals



Mutans streptococci changes at follow-up visits

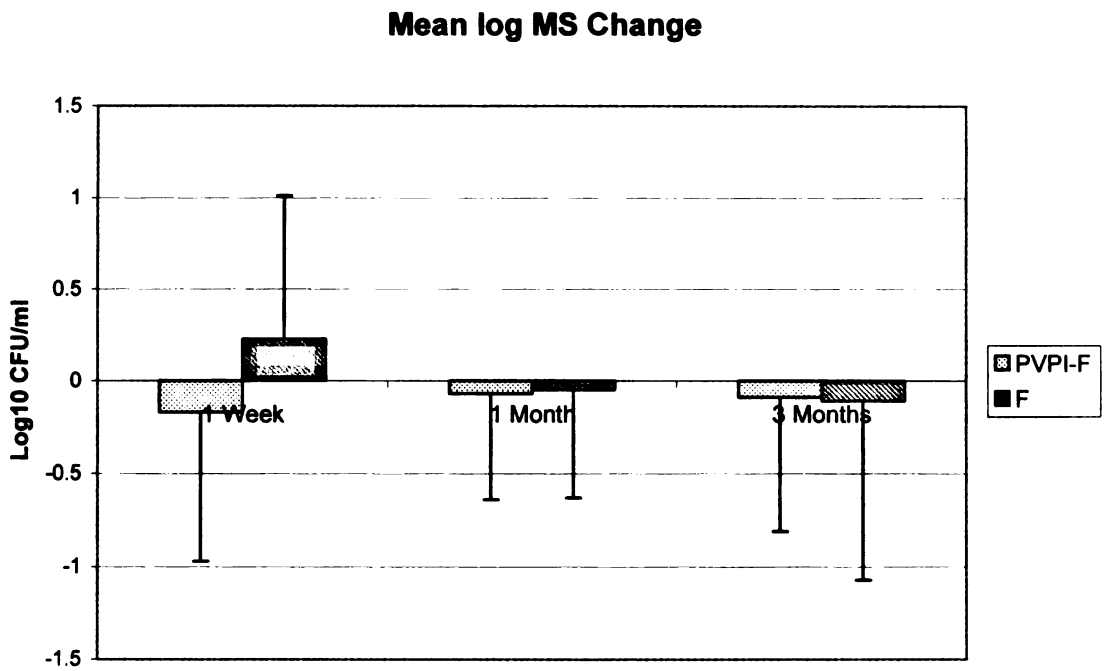
The mean (SD) $\log_{10}$ MS change from baseline at one-week, one-month and three-months is illustrated in Table 4. The $\log_{10}$ MS level at one-week showed a non-significant reduction in the PVPI-F group while a non-significant increase was noted in the F group at one-week. The $\log_{10}$ MS level remained comparable with the baseline level at one-month and three-month visits in both groups. There were no statistically significant differences in $\log_{10}$ MS found between the two groups at one-week, one-month, and three-month visits (T-test,P>0.05).

Table 4 Mean change (SD)* in $\log_{10}$ mutans streptococci levels from Baseline

	PVPI-F	*F*
1 Week	-0.17 (0.80)	0.23 (0.78)
1 Month	-0.07 (0.57)	-0.05 (0.58)
3 Months	-0.09 (0.72)	-0.11 (0.96)

* denotes standard deviation of the mean

Figure 3 Mean change (SD) in $\log_{10}$ mutans streptoccoci levels from Baseline



When the percentage of raw MS counts against raw TVC were calculated and used as a measurement for MS infection levels, MS percentage showed a non-significant reduction at one-week in the PVPI-F group compared to a non-significant increase of MS percentage in the F group. Move over, 90 percent of the subjects in the PVPI-F group had a reduced MS percentage at one-week compared to 27 percent of the subjects in the F group who demonstrated a reduced MS percentage at this time interval. The percent of MS reduction in the PVPI-F group continued to the one-month time interval, but showed an increase at the three-month time interval. The F group showed a reduced MS percentage at one-month and three-month time points. Table 5 shows the changes in levels of mutans streptococci in both the PVPI-F and F groups at one-week, one-month,

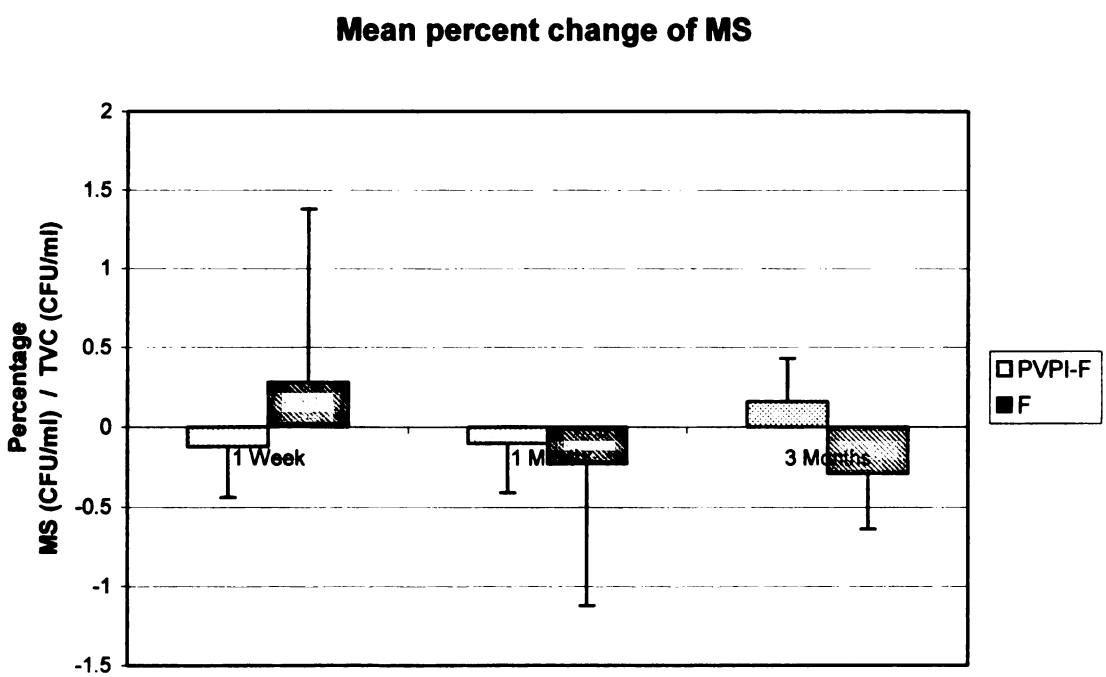
and three-months compared against baseline values. The amount of change from baseline is noted as a percent.

Table 5 Mean change in percent (SD)* of raw mutans streptococci levels against raw TVC

	Mean percent change of MS	
	PVP-IF	*F*
1 Week	-0.12 (0.32)	0.28 (1.10)
1 Month	-0.10 (0.31)	-0.23 (0.89)
3 Months	0.16 (0.27)	-0.29 (0.35)

*denotes standard deviation of the mean

Figure 4 Mean change in percent (SD) of raw mutans streptococci levels against raw TVC



Lactobacilli changes at follow-up visits

Log_{10} LB showed non-significant changes at one-week, one-month, and three-months in the PVPI-F group from baseline. The F group showed no changes in log_{10} LB from baseline at one-week and a non-significant increase in log_{10} LB from baseline at one-month. About a one half log reduction in log_{10} LB was notice in the F group at the three-month interval. It was also found that all subjects in the PVPI-F group with zero log_{10} LB levels remained at zero log_{10} LB for at least one-month after baseline. In contrast, fifty percent of subjects in the F group with zero log_{10} LB at baseline showed LB infection at one-month after treatment. No statistically significant differences were found between the two groups at any visit ($P>0.05$).

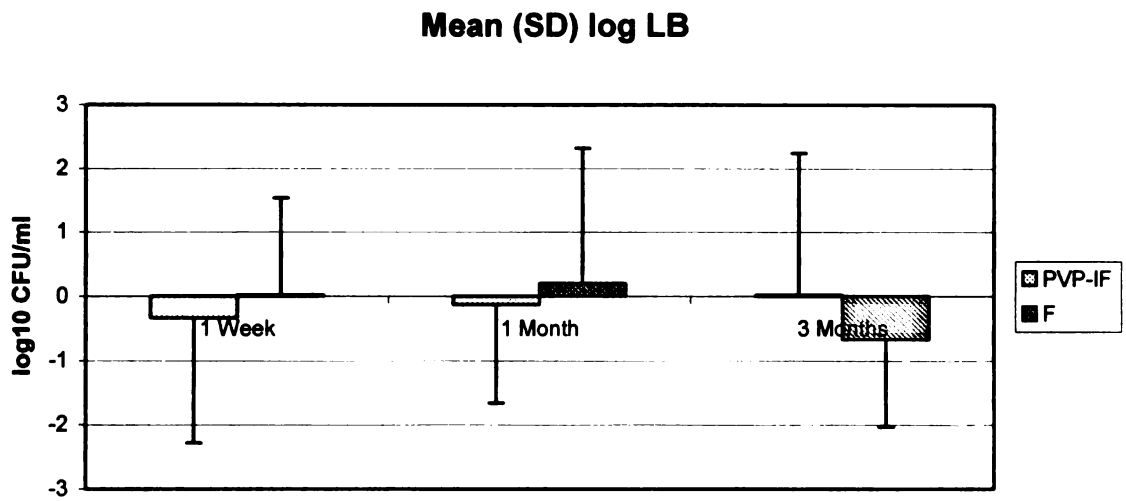
Table 6 demonstrates the differences in lactobacilli levels between the PVPI-F and F groups at one-week, one-month, and three-months as compared to baseline values.

Table 6 Mean change (SD)* in log_{10} LB levels from Baseline

	PVPI-F	*F*
1 Week	-0.34 (1.94)	0.02 (1.51)
1 Month	-0.13 (1.53)	0.20 (2.12)
3 Months	0.02 (2.22)	-0.67 (1.36)

*denotes standard deviation of the mean

Figure 5 Mean change (SD) in log_{10} LB levels from Baseline



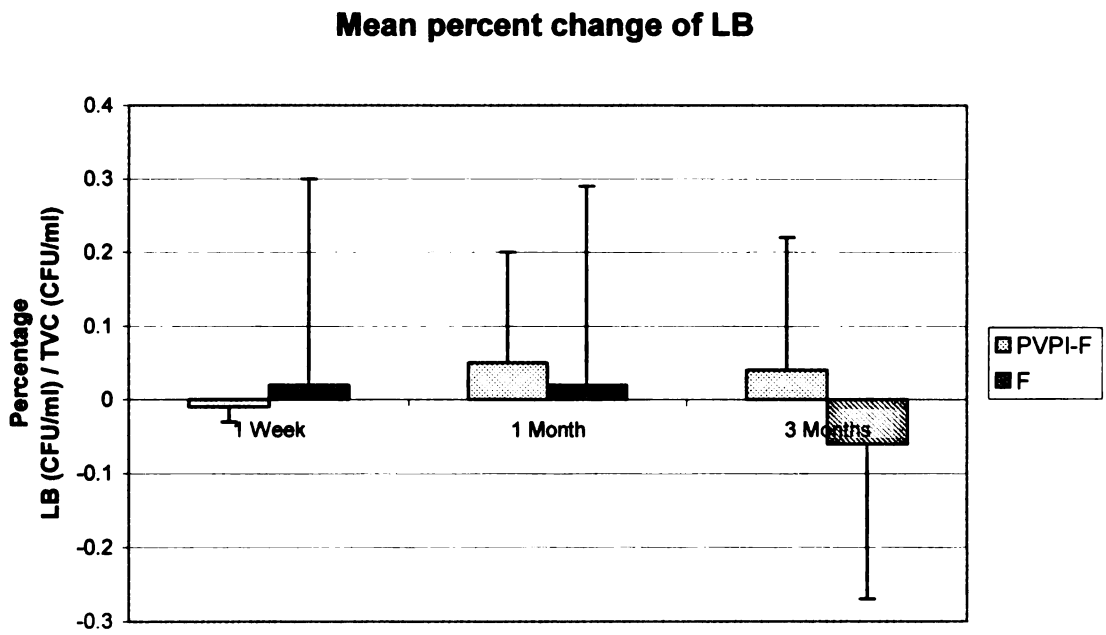
When percentage of raw LB against raw TVC was calculated, it was observed that LB increased at one-week in the PVPI-F group. At one-month and three-months LB percentage increased in the PVPI-F group. In the F group, LB percentage increased at one-week and one-month but decreased at three-months. There were no statistical differences found at any time visit between the two groups (P>0.05). Table 7 demonstrates the percent differences in lactobacilli levels from baseline found at one week, one month, and three months in the PVPI-F and F groups.

Table 7 Mean percent change (SD)* of raw lactobacilli levels against raw TVC

	PVP-IF	*F*
1 Week	-0.01 (0.02)	0.02 (0.28)
1 Month	0.05 (0.15)	0.02 (0.27)
3 Months	0.04 (0.18)	-0.06 (0.21)

*denotes standard deviation of the mean

Figure 6 Mean percent change (SD) of raw lactobacilli levels against raw TVC



Total viable count changes at follow-up visits

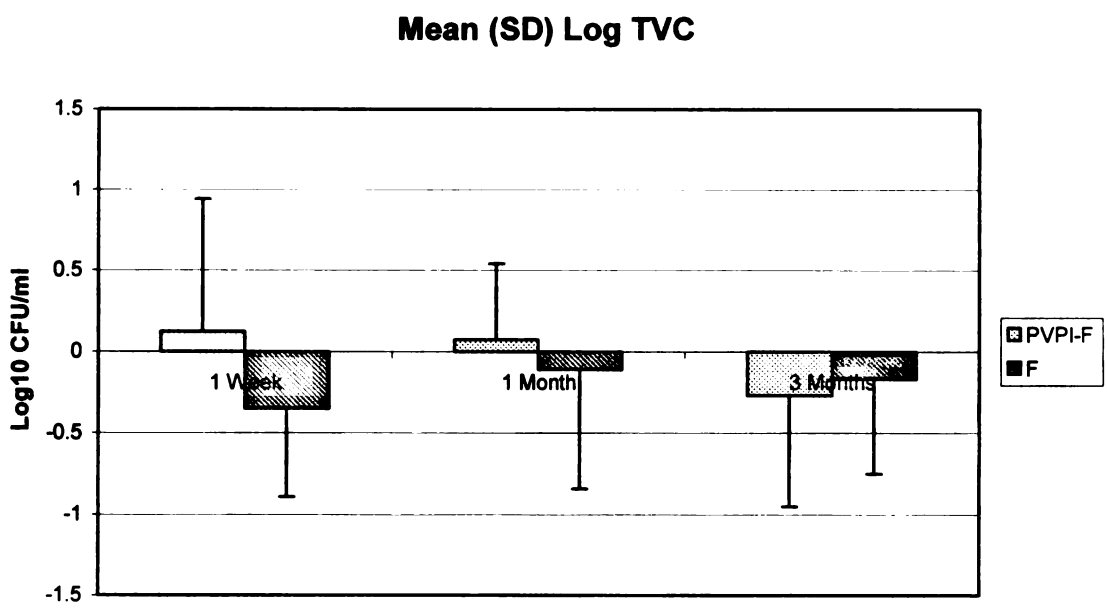
In general, log TVC remained comparable with baseline in both groups although there was a slight decrease at one-week observed in the F group. There were no significant differences in log TVC noted between the two groups at any measured time point (P>0.05). Table 8 illustrates the mean (SD) of total viable bacterial counts in both groups at one-week, one- month, and three-months after treatment.

Table 8 Mean percent change (SD)* in $\log_{10}$ TVC from Baseline

	Mean log TVC	
	PVPI-F Group	*F Group*
1 Week	0.12 (0.82)	-0.35 (0.54)
1 Month	0.07 (0.47)	-0.11 (0.73)
3 Months	-0.27 (0.68)	-0.17 (0.58)

*denotes standard deviation of the mean

Figure 7 Mean change (SD) in $\log_{10}$ TVC from Baseline



6. DISCUSSION

Current methodologies and procedures used on a routine basis for addressing dental disease are limited. Historically, the solution to the problem of dental caries has been to physically remove the portion of the infected tooth and restore the void with a bioinert material. As dentistry evolves, it is becoming more apparent that a new approach needs to be taken in order to address this bacterially mediated disease in a comprehensive manner. This study was designed specifically to contribute to the concerted efforts of many of identifying and evaluating antibacterials and regimens with the potential to reduce cariogenic loads in individuals at high risk for developing dental decay.

Previous studies have supported the possible usefulness of povidone iodine in caries prevention. Caufield and Gibbons showed that after three topical applications of

iodine solution *S. mutans* levels in plaque and saliva could be reduced for 20-24 weeks after treatment.[66] Other studies demonstrated that either I2-KI solution or iodine could decrease detectable levels of *S. mutans*, *Actinomyces*, and lactobacilli in the plaque of occlusal fissure caries by 98%.[67,68]

The present study followed an unpublished study by Fujino and colleagues at UCSF in 2003. In their well-controlled *in vivo* study, children going to the OR for dental rehabilitation were recruited to evaluate efficacy of PVPI. Treatment was administered in the OR immediately following full mouth rehabilitation. Saliva samples were collected one-hour, three-weeks, and three-months after treatment. What they found was that the mean MS and LB levels were significantly decreased at all follow-up visits in the PVPI group.

There are many possible reasons why Fujino and colleagues noted significant MS and LB reductions after PVPI treatment but the present study did not. 1) Povidone iodine was applied to subjects under general anesthesia with little or no saliva present. Saliva may act to dilute povidone iodine or interfere with its attachment to oral structures and ultimately decrease its efficacy. 2) Patient eligibility criteria varied between the two studies concerning subject caries levels. While the previous study identified children referred for general anesthesia (these children usually demonstrate generalized rampant dental decay) as eligible, our study recruited subjects who had either a history of one dental lesion within the past year or current dental decay as eligible. The percent MS and the percent LB density in the previous study, subjects were about 1-1.5 log higher than they were in this study. 3) Subjects from the previous study had full mouth rehabilitation before treatment. Although research has historically supported that removal of dental

infection does not have any effect on overall bacterial loading in the mouth, recent studies by Amin *et al.* have demonstrated that extensive one-time restorative dental treatment results in a significant suppression of *S. mutans* levels at 6 months after treatment.[69] It may be the case that there is a temporary decrease in bacterial loading after rehabilitation as dental decay is removed as well as plaque. It might be that after a particular amount of time without changes to decrease pathologic factors, there are hidden sites that serve as reservoirs for bacteria and they are able to be re-established in the mouth at their baseline levels. In our study we did not control for children receiving dental restorations. There was a significant difference in the numbers of children in each group who had restorations placed as well as a greater difference in the range of restorations that were placed per subject in each group. Only 25% of the PVPI-F subjects received restorations ranging from 1-3 surfaces while 63% of the F subjects received restorations ranging from 1-11 surfaces.

Patient acceptance of the treatment foams was similar. There were two subjects in the F group who complained of foam taste and three subjects in the PVPI-F group who complained of foam taste. Because the PVPI-F foam was flavored with a blueberry flavoring, some of the bitterness of the povidone iodine was probably masked. The study supported or hypothesis that the fruit-flavored PVPI-F can serve as an acceptable treatment regimen for children.

In a recent study by Amin *et al.*, it was found that when povidone iodine was applied three times at two-month intervals upon comprehensive restorative treatment under general anesthesia, the control and intervention groups both experienced significant reduction of *S. mutans* levels six-months after treatment.[69] Interestingly, they concluded

that extensive one-time restorative dental treatment resulted in a significant suppression of *S. mutans* at 6 months.[68]

Similar to Meiers *et al.* and Amin *et al.*, our study also supported reduction of mutans streptococci.[67,69] At one week post-treatment, a single-dose treatment with PVPI-F showed a non-significant reduction of cariogenic bacteria in 80% of subjects in the intervention group.

In this study, there were no statistically significant differences in changes of mutans streptococci levels at various time points in either group. There was a declining trend in $\log_{10}$ MS levels noted in the PVPI-F group at one-week from baseline, but the reduction was not significant. Due to the small sample size at in the PVPI-F group at one-week (n=10), there is a possibility that a significant change of a smaller magnitude would not be detected. When restored ds/DS was analyzed, it was found that there was a statistically significant difference between the number of subjects in the PVPI-F and F groups who received at one-month (Chi-square test, P=0.025). Additionally, there was a statistically significant correlation between a reduction in the percent of mutans streptococci detected at one-month and the amount of restored ds/DS in the control group (Pearson correlation, P=0.01, r=0.55). This data suggests that the placement of restorations does affect mutans streptococci levels over the short-term duration of less than one-month. This finding was not observed in the PVPI-F group, possibly due to the low number of restorations subjects in this group received.

A limitation of this study was the small sample size. Small sample sizes for the one-week follow-up may have masked any significant difference seen between the PVPI-

F group and the F group. Another limitation of the study is not accounting for the role that dental restoration placement could have on our results.

Further research is needed to determine if PVPI-F treatment can significantly reduce mutans streptococci and lactobacilli levels from baseline values if multiple treatments are used. Although the observed initial reduction in bacterial levels was of limited duration, it is still possible that PVPI will be found effective in reducing cariogenic bacterial levels in individuals at high risk for dental decay. Additional research should also explore whether a multiple-dose treatment with PVPI is successful at decreasing cariogenic bacterial levels for a longer duration.

6. CONCLUSION

This study explored the effectiveness of a single-dose administration of povidone iodine against cariogenic bacteria in high caries risk children. While study results fell short of our expectations, there is still optimism that povidone iodine will find an appropriate application in preventive dentistry. This study supports the ability of PVPI-F to reduce $\log_{10}$ mutans streptococci at non-sigificant levels one-week after baseline. A larger sample size is needed to determine if this trend towards reduction can be detected at significant levels. Additionally, an alternative finding of this study is that there was a significant correlation in the F group found between the numbers of restored ds/DS and percent mutans streptococci determined at one-month after baseline. This data suggests that future research should be directed at evaluating multiple-dose povidone iodine regimens in larger sample populations.

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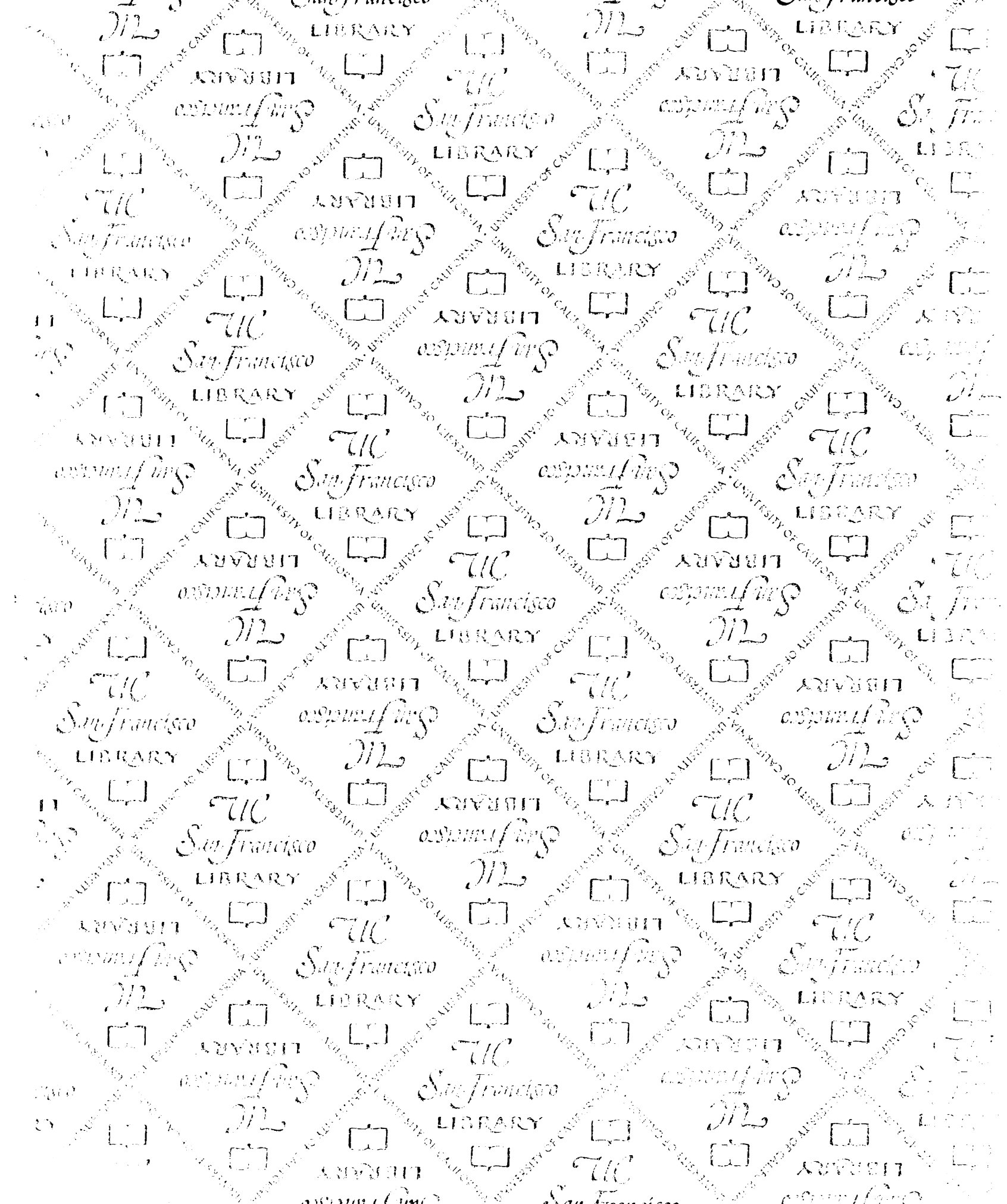
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