EFFECTIVENESS OF A NEW PROTOCOL FOR CARIES
MANAGEMENT IN HIGH RISK PATIENTS

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ABSTRACT

AYESHA SWARN: Effectiveness of a New Protocol for Caries Management in High Risk Patients
(Under the direction of André V. Ritter)

Objective: To (1) examine the effectiveness of a home-care protocol (CariFree®, Oral Biotech, Albany, OR) in reducing caries risk in high-risk adults compared to conventional oral hygiene measures and (2) determine the agreement between a chairside ATP bioluminescence test (CariScreen) and laboratory Mutans Streptococci (MS) counts (CFU/mL of saliva). Methods: 24 high caries risk subjects were randomly assigned to intervention (CariFree treatment protocol) or control (brush twice and floss once daily) groups. Plaque and stimulated saliva samples were evaluated at baseline, 1 and 3 months for ATP bioluminescence, buffering capacity, Lactobacillus and MS counts. Results: At all evaluation times, no statistically significant difference was observed within groups or between the intervention and control group. The CariScreen scores showed poor correlation with MS counts. Conclusions: The CariFree protocol did not reduce caries risk of high-risk adults compared to traditional home-care. There was poor correlation between the CariScreen test and MS counts.
DEDICATION

To my brother
Your strength gives me strength
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1 INTRODUCTION

The global burden of oral disease and dental caries has been steadily rising even as improved and novel tools for reconstructing damaged dentition are rapidly evolving. Approximately 90% of the population in developed countries is affected by this preventable disease[1]. Recent trends suggest an increase in incidence of dental caries amongst specific populations in the US [2]. Dentistry has traditionally managed dental caries through a “drill and fill” approach. This approach has served well to restore function and esthetics, however has failed to prevent the incidence of new carious lesions. The World Health Organization (WHO) through the Sixtieth World Health Assembly has passed a resolution that advocates integration of prevention and early intervention measures of dental caries for all the member nations [3]. Over the past decades, the health care organizations in many nations have still not implemented mandatory comprehensive caries prevention into routine dental practice [4]. The increased burden of the disease worldwide may also be attributed to the fact that dentists as a majority both in dental schools and practices still focus on the treatment rather than prevention of the disease.

Caries prevention and treatment based on an analysis of the presence or absence of risk factors is advocated by the American Dental Association. Caries risk assessment through risk indicators (diet, host and microbiological) was first elaborated upon in a supplement by JADA in 1995 [5]. An accurate, valid and efficient caries risk assessment
model must consistently identify high caries risk patients. These models base the caries risk analysis on a number of factors—both clinical and biological. Caries management by risk assessment (CAMBRA) is a system that focuses on treating and preventing the cause of the disease at an early stage, rather than waiting until it causes irreversible damage to tooth [6-8]. This system is being advocated as a standard of care for practicing dentistry within the dental community.

Studies comparing the appropriate preventive and interventional protocols for caries management following a caries risk assessment have been used for the validation of caries risk assessment models. A systematic review conducted on selected caries prevention methods concluded that enough evidence does not exist to determine the efficacy of new methods of caries prevention and management[9]. A recommendation has been made to increase the number of studies examining prevention among high risk individuals.

The purpose of this thesis is to compare a new protocol for caries management (CariFree®, Oral BioTech, Albany, OR) to conventional oral hygiene measures for treatment of high caries risk subjects. CariFree is a new caries assessment and treatment model based on CAMBRA and uses fluoride, xylitol and pH alteration for decreasing the individual’s risk for caries [10, 11].

The organization of this thesis is as follows. A description of the Objectives of this research is followed by a summary of relevant literature. The Materials and Methods section follows the review and describes the study design, materials used and data collection instruments and sequence. The Results and Discussion sections illustrate the
outcomes of the study followed by a section highlighting the Conclusions drawn from the study outcomes.
2 OBJECTIVES

The purpose of this study was to examine the effectiveness of a new protocol for caries management in reducing caries risk when used in high risk adults. The treatment protocol consisted of using home care oral hygiene products (CariFree) for a period of 90 days. The study hypothesis was that the CariFree Treatment Protocol is more effective in reducing caries risk than conventional oral hygiene measures after a 90 day regimen in high risk adults.

2.1 Specific Aims

2.1.1 Primary Aim

To examine the effectiveness of a new home care protocol in reducing caries risk in high risk adults when compared with conventional (or usual) home care.

2.1.2 Secondary Aim

To determine the agreement between a chairside ATP bioluminescence test (CariScreen) and a laboratory-based salivary Streptococcal Mutans count (CFU/mL of saliva).
2.2 Hypotheses

Primary Aim: The use of CariFree home care protocol will reduce the caries risk in high caries risk adults after a 90 day regimen in comparison with those continuing with conventional oral hygiene practices.

Secondary Aim: A good agreement exists between the ATP bioluminescence test and Streptococcal mutans counts.
3 LITERATURE REVIEW

Research over the past 50 years has established dental caries as a bacterial based chronic infectious disease. It presents with a time dependent complex etiology involving tooth substrate, bacterial flora, presence of a fermentable carbohydrate source, and access to minerals namely calcium, phosphate and fluoride. The bacteria present in the plaque or oral biofilm adherent to the tooth surface ferment carbohydrates contained in our diet and release acids. These acids diffuse into enamel and dentin, dissolving the mineral components of the tooth. Under normal conditions the mineral lattice of the tooth behaves like a sieve and free ions such as Ca$^{++}$ and PO$_4^{-}$ present in the saliva migrate back into the lattice of the tooth once the acid is buffered by the saliva.

While this process of demineralization and remineralization is constantly occurring underneath the oral biofilm, under special conditions, a sub-surface carious lesion or break in the tooth surface can develop. These special conditions or risk markers are numerous and may include presence of acidogenic bacteria, reduced salivary function coupled with frequent acid attack, and presence of oral restorations or devices promoting biofilm build-up [12]. The following review attempts to look at the current concepts and known risk markers for caries and how they interplay with one another to determine the caries risk of an individual. The current accepted treatment protocols for managing patients with high caries risk shall also be reviewed subsequently.
3.1 Caries Disease Process

Specific strains of bacteria such as Mutans Streptococcus (MS) and Lactobacilli (LB), associated with the initiation and progression of the disease, have consistently been isolated in oral plaque and salivary samples. These two strains MS and LB are aciduric and acidogenic with pH varying from 3.8-4.8. For many years it has been considered that the low pH products of metabolism from these two strains result in acid dissolution of tooth structure and further progression of disease [4, 13-15]. More recent research have suggested that while these two strains may be considered as markers for the disease, there are additional groups of organisms that are involved with the progression of the disease[15, 16]. Since the traits associated with cariogenicity are not associated with a single species of bacteria, it is but evident that measures to combat caries initiation and progression must include other intervention factors.

Caries must be viewed as a disease resulting from changes in the ecology of bacterial communities in the mouth. The Keys-Venn diagram (insert figure) depicting the etiology of caries has been modified now to include the ecological plaque hypothesis [17]. Change in the environment such as carbohydrate intake and xerostomia can lead to these dramatic shifts in oral environment. Many studies report the correlation between increase in bacterial flora and presence of disease; however the balance between environment, plaque microflora and integrity of tooth structure is yet to be investigated completely [15, 16, 18, 19]. Bowden in 1984 posed pertinent questions regarding the nature of the environmental stresses that convert a healthy biofilm into a pathogenic
biofilm [20]. The additional questions arising and yet unanswered entirely are whether the modification of the biofilm environment reduces caries.

Dental plaque consists of a film of polymers and bacteria over the tooth surface. These bacteria under stress team up and form a collective called oral biofilm which is attached to the tooth surface. Andre Levchenko mentions that biofilms are large communities organized like cities with channels for nutrients to go in and waste to go out [21]. The role that Mutans Streptococcus and other organisms present in biofilms play is continuing to unfold methodically through dedicated research. Genetic analysis using genotypic methods such as 16S rRNA gene sequence analysis are being increasingly employed for the isolation of potential pathogens [22]. Interestingly, biofilm growth has shown both indirect and direct influences on gene expression by oral bacterial [23].

Other important virulence factors being investigated are the Streptococcus Mutans Glucosyltransferases. The glucans produced by this enzyme released from the Streptococcus Mutans helps more bacteria to adhere to the tooth surface. The molecular structure of glucans changes when glucosyltransferases are adsorbed by a surface [24]. Additionally, other oral bacteria also start to produce glucans once exposed to this enzyme [25]. The multiplication of bacteria and subsequent formation of a complex extracellular matrix results in a stable and resistant shield around the biofilm. This plays a critical role in preventing exogenous pathogenic species from colonizing the mouth (colonization resistance) [26]. Bacteria growing in the biofilm also start developing resistance to anti-microbial agents [27]. In 1996 it was demonstrated that a 24 h plaque sample required multiple times the minimum concentration of chlorhexidine to eliminate S. Sanguinis, and an even higher concentration is required by a 72 h sample [28]. The
reasons for the increased biofilm tolerance to antimicrobials were elaborated upon by Stewart et al. They hypothesized poor antibiotic penetration, nutrient limitation and slow growth, adaptive stress responses, and formation of persister cells as reasons for the heightened antibiotic resistance [29]. Other confounding features of the biofilm include the heterogeneous distribution of the pH within the structure of the biofilm [30]. This facilitates the growth of different genera of bacteria within the community, which otherwise do not coexist. Consequently an extremely tenacious bio-community impervious to antibiotics and permanent disruption develops around teeth in susceptible individuals. Meanwhile, the persistent aciduric and acidogenic colonies present in the underbelly of this multilayered film, continue to cause acid demineralization of the tooth structure in the absence of the protective functions of saliva.

The process of demineralization of the tooth by the aciduric and acidogenic bacteria in the biofilm can be arrested by the presence of saliva containing acid buffers and antimicrobial enzymes in the initial stages [19, 31, 32]. However, continued acid attack coupled with poor oral hygiene and dietary habits could result in active disease. Both quality and quantity of saliva is important to maintain the oral structures. Since cariogenic substances are constantly introduced into the oral cavity, saliva aids in diluting the acid and sugars and providing oral clearance. Sites in the mouth closer to orifices of salivary ducts have a faster clearance rate compared to other areas like mandibular buccal surfaces making them more susceptible to biofilm colonization. The residual volume of saliva left over after swallowing forms a thin film averaging around 0.1mm thick and develops a concentrated reservoir of sucrose after a meal. This varies based on location, tooth morphology and sex of the patient [33]. A correlation has been noted between the
salivary clearance of a patient and the pH changes of plaque. The Stephan Curve describes the change in dental plaque pH in response to a challenge. The slopes of Stephan curves vary for slow and fast clearance rates [33]. As the pH of the biofilm becomes more acidic, only those bacteria that can survive in a low pH grow. These bacteria are responsible for the demineralization of teeth and the caries process. Bradshaw, McKee, and Marsh in 1989 described the effects of carbohydrate pulses and pH on population shifts within oral microbial communities [34]. The results of their experiments demonstrated that in presence of sugar, the SM and LB species multiplied only in the media in which pH changes were allowed to occur (Figure 1). Hence control over the plaque pH through efficient buffering would seem key in preventing the caries process.

Salivary gland hypofunction is also contributory towards caries risk. While hyposalivation is an objective measure of the amount of salivary production and xerostomia is a subjective feeling of dry mouth, both terminologies are often used in place of each other erroneously. Average normal values of salivary flow rates are 1.5ml/min stimulated and 0.3ml/min unstimulated. However these values have a large variation based on age and sex. To be classified as abnormally low, the salivary flow rate for saliva must be equal to or less than 0.1ml/min for unstimulated and 0.5-0.7ml/min for stimulated. Nonetheless, the symptoms of dry mouth may be experienced even at higher salivary rates. Some of the common causes of hyposalivation include medication, radiation therapy, immune deficiencies, and salivary gland inflammations.

Quality of saliva is dependent on the protein content, viscosity, pH and buffering capacity. Saliva carries antifungal and antibacterial peptides; calcium, phosphate and
fluoride ions for potential remineralization of tooth and lubricants to keep mouth moist[35]. While buffering capacity of saliva is often employed as a measure of caries risk, the measure is not a precise assessment of the pH of the microflora within the depths of the biofilm.

3.2 Caries Risk Assessment and Management

Evidence based protocols for risk assessment and management of dental caries has gained momentum amongst the dental community in recent times [7, 36]. Caries risk assessment has over the past decade included a two pronged approach-evaluating populations and individualized risk assessment [37]. The disproportionate distribution of caries by race, age, geographic area and socioeconomic status submits the need for individualized caries risk assessment [38]. Caries predictive models for pre-school and school age children have been reported [39, 40]. A review of microbiological factors concluded that while specific bacteria may not be a reliable risk indicator for caries risk for individuals but might be useful while evaluating high caries risk groups [41]. The review goes on to suggest that SM and LB counts may be more predictive in establishing low caries risk more consistently than high caries risk. The past caries experience of a patient has been adjudged as the variable with highest predictive value by others [36, 42]. Due to the multifactorial nature of caries etiology, it is expected that multivariate approaches rather than the use of single parameters may improve caries risk prediction for individuals as well as groups of subjects.
Various attempts to group the etiological agents of the disease and caries risk predictors have been made in the past. For the purpose of this review, the causative factors which are predictive of disease but not necessarily etiologic variables are referred as “risk indicators”. Other terminologies include “risk factors” as causative variables. A recent review of the clinical studies looking at risk predictors for adult and adolescent dentition and out of 24 total studies found that all included past caries experience as an important predictor followed by “other variables”. Microbiology and host factors completed the list in both adults and children. While looking at root caries, the host factors were ranked higher than the microbiology. So the challenge is to better define how contributing factors interact and how this translates into a cost-effective strategy for disease diagnosis, prevention, and management. In a recent review on the caries risk assessment for prosthodontic patients, a tabulation of the most common risk factors based on clinical exams were presented [8]. Tables 1 and 2 present an edited version of the same.

The validity of the various caries prediction models that are present to predict caries need to be studied in the context of their application within specific populations. All models are typically arithmetic calculations including a combination of the above mentioned risk factors and indicators. A high specificity for the model is desirable in case the instrument is used for a large population based survey in order to avoid false positives. Similarly for individual analysis it becomes critical to have a high sensitivity to reduce false negatives. Based on the assessments, the subjects are classified as high, intermediate and low risk and the treatment protocols are then determined.
3.2.1 CAMBRA (Caries Management by Risk Assessment)

CAMBRA was formed by an unofficial group called the Western CAMBRA Coalition and included representatives from education, research, industry, governmental agencies and private practitioners based in the western states of the US [43]. The CAMBRA risk assessment tool divides risk of developing dental caries into the following subdivisions [7].

3.2.1.1 Caries risk indicators

Visible cavities or radiographic penetration of the dentin, radiographic proximal enamel lesions (not in dentin), white spots on smooth surfaces, restorations last 3 years.

3.2.1.2 Caries risk factors (Biological predisposing factors)

Mutans Streptococcus (MS) and Lactobacillus (LB) both medium or high (by culture), visible heavy plaque on teeth, frequent snack (> 3x daily between meals), deep pits and fissures, recreational drug use, inadequate saliva flow by observation or measurement saliva reducing factors (medications/radiation/systemic), exposed roots, orthodontic appliances.
3.2.1.3  *Caries protective factors*

Lives/work/school fluoridated community, fluoride toothpaste at least once daily, fluoride toothpaste at least 2x daily, fluoride mouthrinse (0.05% NaF) daily, 5,000 ppm F fluoride toothpaste daily, fluoride varnish in last 6 months, office F topical in last 6 months, chlorhexidine prescribed/used one week each of last 6 months, xylitol gum/lozenges 4x daily last 6 months, calcium and phosphate paste during last 6 months, Adequate saliva flow (> 1 ml/min stimulated).

Figure 2 represents the interplay between the various factors. The caries balance must be maintained by appropriate use of therapeutic agents since surgically replacing the diseased part of the tooth does not decrease risk of future disease [44, 45]. The past experience of the disease itself being its most prominent risk indicator, an emphasis on a preventive approach to treat the disease needs to be investigated further. Since dental caries is a biofilm (or bacteria) mediated disease, the current evidence-based prevention methods include fluoride applications, diet modifications and good oral hygiene practices.

3.3  Motivational Interviewing, Diet Modification, and Oral Hygiene Practices

Sugar plays an important role in the initiation and progression of the carious disease process. A thorough diet analysis to isolate harmful eating patterns such as sugary drinks with low pH, frequent snacking and inadequate oral hygiene measures needs to be performed prior to initiating a caries reduction protocol and expensive restorative
therapy. Behavioral patterns need to be reviewed. Patient compliance is often the biggest factor reducing the success of caries control therapy [46]. Motivational interviewing, a patient-centered counseling technique, has been found to be effective in reducing caries in high-risk young children [47]. It was first described by Dr William Miller in 1983 in relation to his work with alcoholics. The approach includes techniques such as open-ended questions, reflective listening, affirmation, and summarization to help individuals express their concerns about change. Evidence of success using this technique in the health care setting is useful in improving patient adherence to treatment protocols.

Primary causative agents for caries being the resident oral flora, the goal of therapy is to reduce or control the number of bacteria and not eliminate the flora completely. Mechanical removal of plaque by itself has documented to be inadequate without use of adjunct chemical methods.

3.4 Chemical Agents

Most antimicrobial agents used against plaque can be separated on the basis of their mode of action. The primary modes are by inhibition of microbial colonization, inhibition of microbial growth, disruption of mature plaque and modification of plaque biochemistry and ecology[33]. A review of all the chemical agents available is beyond the scope of this document, but a few agents with a building evidence base in efficacy against caries shall be reviewed.
3.4.1 Fluoride

The evidence base for fluoride significantly decreasing caries risk is well established [48, 49]. The mode of action of these products is via fluoroapatite formation, remineralization, antimicrobial action and prevention of ionic bonding of pellicle to tooth. This effect is however based on the patients overall burden of risk factors. Fluorides are available for topical applications mouthrinses, gels, or varnishes; dentifrices, or systemic through milk and water fluoridation. Reports in literature suggests that topical fluorides used in addition to fluoride toothpaste achieve a modest reduction in caries compared to toothpaste used alone [50]. Toothpastes containing fluoride between 1000-1100 ppm have proven effective while anything below 600ppm have shown to be of limited value. The overall reduction in caries reported with their use is between 20-35% [51]. Fluoride containing oral rinses when used along with brushing twice daily with fluoridated toothpaste provided an increase in caries reduction by 10%. While both the above mentioned forms of fluoride delivery depend on patient compliance, professional application of the fluoride at regular intervals as varnishes (5% NaF) have proven to be efficacious [48, 52].

Fluoride containing products along with pit and fissure sealants and dietary management are considered as the mainstay of caries management. Other non-fluoride containing products are also available in the profession. The American Dental Association (ADA) Council on Scientific Affairs issued a report in 2011 containing clinical guidelines for non-fluoride caries preventive agents. The expert panel's recommendations were based on a review of evidence from 71 published articles that described 50 randomized controlled trials and 15 nonrandomized studies assessing the
effectiveness of various non-fluoride agents in preventing cavities. The review included the current available agents such as polyol sugars, chlorhexidine, arginine and probiotics. A brief review of these agents follows.

3.4.2 Xylitol

Xylitol by chemistry is a “polyol” or “sugar alcohol” (Figure 3). Xylitol along with sorbitol are non-fermentable sugars and are used as a sugar replacement in chewing gums.

The regular use of xylitol as mints or chewing gums is suspected to prevent caries by increasing salivary flow through mastication, reducing colonies of MS and reducing plaque acidogenesis [53]. This altered pH of the environment possibly aids in promoting remineralization of subsurface enamel lesions. Xylitol also has the unique ability to select for a MS population with weakened virulence factors [54, 55]. Recommended doses are 5-7 grams of xylitol at a frequency of at least three times per day [56], but a lack of consensus however regarding their dosage still exists. Additionally, the primary therapeutic agent is available in many marketable forms such as sugar-free chewing gum, lozenges and hard candy including xylitol or polyol combinations [57]. The ADA recommends 10 to 20 minutes of chewing of sucrose free polyols after meals may prove as adjuncts to reduce incidence of coronal caries.
3.4.3 Chlorhexidine

Chlorhexidine (CHX) has a well-established evidence base as an antiplaque agent and is used as a gold standard of treatment. It was first introduced in 1954 as an antiseptic. CHX is a strong base with cationic properties and binds to the negatively charged bacterial wall causing disturbance of membrane functions. It is effective on both gram positive and gram negative bacteria. The effect on gram negative bacteria however is weak. The chemical agent is bacteriostatic in strong concentrations and immediately after application. Literature supports reduction in plaque microflora by 80-95% on single mouthrinse with 0.2% CHX solution. It also helps convert the pH of the plaque by retarding the metabolism of bacteria in low doses by inhibiting bacterial enzymes such as glucosyltransferase and phosphoenolpyruvate phosphotransferase [58]. CHX displays very high substantive properties and maintains bacteriostatic potency even after adsorption on tooth surface. Currently, only mouthrinses containing 0.12 percent CHX are marketed in the United States (US). Two independent reviews of literature concluded that the evidence behind efficacy of CHX rinses in reducing caries has been inconclusive [59, 60]. Hence CHX rinses alone cannot be recommended for caries control. A synergistic effect between fluoride and CHX has been documented on some high risk adult populations in literature [61]. However, due to opposite charge on the ions, they must be used at least 1 hour apart. CHX is also available in a varnish formulation. The expert panel at the ADA Council for Scientific Affairs has found that a 1:1 mixture of chlorhexidine/thymol varnish may be efficacious in the prevention of root caries [62]. More evidence is required regarding application frequency and long term effect after last application.
3.4.4 Sodium Hypochlorite

Sodium hypochlorite solution is frequently used as a disinfectant or a bleaching agent. It has broad antimicrobial activity, rapid bactericidal action, relative non-toxicity at use concentrations and no staining. Hypochlorite is lethal to most bacteria, fungi and viruses. Hypochlorite solutions are extremely reactive and gradually lose strength, so fresh solutions should be prepared daily. At high concentrations however, irritation of mucous membranes is noticed. Hypochlorite is also known to have deproteinization effect and has shown to increase the level of Ca\(^{+2}\) uptake by carious lesions in experimental in-vitro specimens [63]. More evidence regarding the anti-cariogenic effect of sodium hypochlorite is necessary at this time to validate its use as an effective anti-cariogenic agent.

3.4.5 Arginine

Recent research has shown the addition of the arginine within dentifrices and food products interferes with the initiation and progression of caries [64]. Tooth remineralization has also shown to be positively stimulated. The interaction of arginine with other flora at this time needs to be investigated further before a definitive disease reduction benefit can be observed.

3.4.6 Probiotics and Genetically Modified Bacteria

Probiotics are usually live microrganisms (bacteria) which are similar to naturally-occurring oral bacteria. These probiotics are currently under investigation since
their safety and effectiveness long term have not been tested satisfactorily. Genetically modified biomolecules aimed at preferentially targeting cariogenic species in biofilms have also been engineered. Ongoing research in these “smart molecules” against specific bacteria are encouraging; however further investigation is necessary before they can be introduced in the population [65, 66].

3.4.7 CariFree®

CariFree (Oral BioTech, Albany, OR) is a caries risk assessment and treatment model based on the CAMBRA approach. Caries risk is determined based on a questionnaire and a chair-side measurement of the adenosine triphosphate (ATP) bioluminescence (CariScreen Caries Susceptibility Testing) from the plaque present on specific sites within the oral cavity. The ATP-driven bioluminescence assays have long been used as a quantitative measure of microbial numbers in the packaged food industry and more recently for measuring total bacterial mass in dental plaque [67, 68]. Based on the level of caries risk, the patient is placed on a treatment regimen. This do-at-home treatment includes oral rinses, toothpaste substitutes and chewing gum containing the benefits of xylitol, fluoride and pH neutralizing agents. These specific agents help to modify the salivary environment and build resistance against acid attack. The CariScreen Caries Susceptibility Testing meter can be used chair side and is a validated tool [11, 69].
4 Materials And Methods

This study was a prospective randomized controlled clinical trial to evaluate the effectiveness of the CariFree Treatment protocol in reducing caries risk markers on high caries risk individuals. During the screening process, subjects were categorized as at risk or low risk on the basis of a Caries Risk Assessment (CRA) form and an ATP bioluminescence score (CariScreen). Subjects were randomized and placed into either the intervention group (receiving CariFree products) or the control group (continued conventional oral oral hygiene practices) and followed for 90 days to observe the change in their caries risk based on a set of predefined outcome variables, or caries risk markers. Saliva and plaque samples were collected at 3 time periods; baseline (visit 1), 30 days after baseline (visit 2) and 90 days after baseline (visit 3).

The research protocol was reviewed and approved by the Institutional Review Board (IRB, study #10-1529) of the University of North Carolina at Chapel Hill. Figure 4 provides a simplified overview of the study design.
4.1 Participant Selection

All the participants selected for this study (n=24) were recruited from the UNC School of Dentistry Clinics patient pool and UNC student/employee population. Potential participants were only approached after IRB review and approval of the study protocol, consent forms, and other documents. IRB authorization (and waiver) to access clinic schedules and pre-screen patient records in advance of clinic appointments was also obtained, so that potential subjects that satisfy inclusion criteria and showed evidence of recent restorative work could be approached and invited to participate. Patients were screened at the UNC School of Dentistry clinics and only high caries risk subjects were recruited. Inclusion and exclusion criteria were as follows:

**Inclusion criteria**

- Adults (18-80 years old)
- At least 12 teeth present
- At risk (based on CariScreen scores > 1500 and CRA form)
- Agreement to comply with study protocol
- Able to read, understand, and sign consent form

**Exclusion criteria**

- Allergy to study materials components
- Periodontal disease Type IV
- Undergoing antibiotic therapy
- Undergoing radiation therapy
- Subjects participating in any other caries interventional studies
A chair-side ATP bioluminescence measurement (CariScreen) and a caries risk assessment analysis (CRA form) were performed on all qualifying subjects willing to participate in the study. The first 24 subjects with a combined “at risk” reading with CariScreen and CRA form were enrolled. Details about using the tools and interpreting the reading are provided in the following sections.

4.1.1 Caries Risk Assessment Form (CRA)

The CRA form (Appendix A) is a simplified version of the risk assessment form developed by at the University of California, San Francisco. It has been further modified by others to make it less time consuming for the dental practice setting [7, 45]. The form covers disease indicators such as radiographic lesions and white spot lesions along with such risk factors as diet, dental hygiene, saliva flow, medications, bacterial population, and dental history to identify risk for decay. A subject was categorized as “at risk” with a CRA score of 1 or more risk indicators and 2 or more risk factors.

4.1.2 CariScreen Caries Susceptibility Testing

4.1.2.1 The CariScreen Caries Susceptibility Testing Swabs

Plaque sample was obtained by using a sterile swab (Oral BioTech). The pre-packaged swabs were received in a batch of 25 tips. They were stored at 2-8°C, and maintained at room temperature for 15 minutes prior to the measurement. The swabs contain the bioluminescence reactive agents in a partition bulb which is released by
physically breaking the seal after swabbing. The reactive agents combine with the plaque bacteria and a chemical reaction occurs. Chemical energy is converted to light energy once placed in the CariScreen Caries Susceptibility Testing Meter and the result is read as the intensity of light in Relative Light Units (RLU). The test must be read within 1 minute of activating.

Reaction [69]:

$$\text{ATP} + \text{Luciferin} + \text{O}_2 + \text{Luciferase} + \text{Mg}^{2+} \rightarrow \text{AMP} + \text{oxyluciferin} + \text{PPi} + \text{CO}_2 + \text{light} ((560 \text{ nm})$$

4.1.2.2 The CariScreen Caries Susceptibility Testing Meter

The meter is a hand held device used to measure the chair-side ATP obtained from a sample of the subject’s plaque mass. The meter needs to be calibrated internally each time before making a measurement. This is done by turning it on and takes 60 seconds to be ready to receive a fresh swab sample.

The procedure for making the measurement is described below and was performed as per the manufacturer’s recommendations.

4.1.3 Plaque Sample Collection

After calibrating the meter, the swab was removed from its protective tube and held close to its tip. A single swipe was made across the lingual surface of the mandibular anterior teeth (#22- #27) avoiding contact with soft tissue (Figure 5).
4.1.3.1 Sample activation

The swab tube is secured with one hand, and the thumb and index fingers of other hand are used to snap the valve connecting shaft to bulb containing the enzymatic liquid. The liquid is squeezed into the tube shaft and allowed to bathe the swab for 10 sec. The swab is now activated and must be read in the meter within 1 min.

4.1.3.2 Meter reading

The activated CarieScreen device is inserted in to the CarieScreen meter and the lid is shut. The device is turned on and counts down till 15 sec. Readings appear on a screen in the form of Relative light units (RLU). The luciferase contained in the CariScreen system is based on the “flash-type” luminescence signal, with RLU readouts peaking at 2 minutes [69]. According to manufacturer’s guidelines they are interpreted as: “low risk” 1-1500; “at risk” 1501-9999. This was done within 1 minute of swabbing for all samples collected. The swab was then removed and destroyed.

4.2 Subject Allocation and Randomization Schedule

Once the subject was considered high risk and accepted into the study, all the necessary consent and HIPAA forms were reviewed and signed. A stratified block randomization schedule was used to assign subjects to intervention or control arms in a parallel group design with a 1:1 allocation. A permuted block design (Table 3) developed
with a computer random number generator ensured that equal numbers of subjects (n=12) were assigned to each arm. This is critical in trials with small numbers of subjects. The purpose of randomization was to balance the arms as much as possible with respect to known and unknown prognostic factors for dental caries. Within blocks, stratification ensured that both groups were balanced on salivary pH (low versus high pH), an important characteristic influencing dental caries risk. The salivary pH was measured by placing pHion Diagnostic pH Test Strips (pHion Balance, Scottsdale, AZ) in the buccal vestibule for 15 seconds. The chairside pH was determined by color transition of the strips and read as numerical scores in increments of 0.25. Those subjects 6.5 and above were considered low risk and those below were considered as high risk. Overall, this design optimized conditions to test the efficacy of the CariFree intervention on risk of dental caries.

The intervention group received the CariFree Treatment products to be used for 3 months as per manufacturer recommendations and the control group continued oral hygiene practices. Both protocols are described in detail in the following sections.

4.2.1 CariFree Treatment Protocol

The 12 subjects in the intervention group were provided with a treatment kit along with verbal and written instructions. The protocol is as follows:
4.2.1.1  *CariFree Treatment rinse*

This rinse is used in the first 30 day of starting treatment. The rinse consists of sodium fluoride 0.05%, water, xylitol, menthol, natural flavors, sodium benzoate, poloxomer 407, sodium hydroxide, sodium hypochlorite. Instructions include using rinse for two times daily for 30 days. Instructions- Mix 5mL from bottle A with 5ml from bottle B and rinse with solution for 1 minute and spit out. Wait 30 minutes before eating or drinking.

4.2.1.2  *CariFree Maintenance rinse*

The maintenance rinse is used after the 30 day use of the treatment rinse. It contains sodium fluoride 0.05%, menthol, natural flavors, polysorbate 20, potassium sorbate, sodium benzoate, sodium bicarbonate, water, xylitol. It must be used twice daily after brushing and flossing for 60 days. Instructions- Rinse with 10mL of the solution for 1 minute and spit out. Wait 30 minutes before eating or drinking.

4.2.1.3  *CarieFree Oral neutralizer gel*

The gel contains glycerin, hydrogenated starch hydrolysate, hydroxethyl cellulose, menthol, natural flavors, polysorbate 20, potassium sorbate, sodium benzoate, sodium bicarbonate, sodium hydroxide, sodium lauryl sulfate, water, xylitol. Instructions- Use twice daily as a toothpaste supplement for 90 days.
4.2.1.4 *CariFree Boost*

Boost is an oral spray with a pH close to neutral. It contains purified water, xylitol, glycerin, sodium benzoate, calcium hydroxide, natural flavors, and natural colors. Instructions- Use 2-3 sprays in mouth as often as needed to relieve dry mouth and neutralize acids. Between meals and before bedtime recommended.

4.2.1.5 *CariFree Xylitol gum*

A small sample (6 parts) of chewing gum was included. This did not constitute the center of the intervention treatment, but was used more to introduce patients to a xylitol based chewing gum. It contains xylitol, gum base, natural flavors, glycerin, gum arabic, soy lecithin, calcium acetate, beeswax. Instructions- Chew 2 pieces, 3-5 times daily. Recommended after meals or when dry mouth/bad breath occurs.

4.2.2 *Control group*

The 12 subjects in the control group were asked to follow conventional oral hygiene practices for 90 days. This constitutes brushing 2 times daily with an over the counter fluoridated tooth paste and flossing once daily.

Subjects in both the intervention (n=12) and control group (n=12) had sample collection at baseline (visit 1) for their ATP bioluminescence scores and stimulated saliva. The measurements were repeated at day 30 (visit 2), and day 90 (visit 3) from the baseline records.
4.2.3 Stimulated Saliva Collection

For the saliva collection, the participants were given a paraffin wax tablet and instructed to chew on it for 5 minutes and saliva was collected and expelled into a sterile calibrated collection container up to the 5 minute mark. All samples were collected at the same time of the day for each subject. The sample was coded with the unique participant identifier and transported on ice to the laboratory for determination of buffering capacity, the mutans streptococci (MS) and lactobacilli (LB) counts. The saliva was diluted four-fold in 0.005N HCl and the final pH determined after ten minutes. The pH values of 4.0 or less were considered high risk, 4.1 to 4.9 intermediate risk and 5.0 or greater were considered normal. Serial 10-fold dilutions of saliva were done and quantitatively plated (Spiral Plater™ model DU2; Spiral Systems Inc.) to Difco™ Mitis Salivarius Agar supplemented with Chapman Tellurite solution and with bacitracin for the selective enumeration of MS and to BBL™ LBS Agar for the selective isolation and enumeration of Lactobacilli (Figure 6). Counts were performed after 48 hours on a ProtoCOL RGB model no. 90000 (Microbiological International Inc.). The limit of detection for bacterial counts was 204 CFU/mL of saliva. MS counts less than $10^4$ CFU/mL and for LB less than $10^3$ CFU/mL were considered normally healthy. Total saliva and flow rates were also recorded. All samples were destroyed after testing.
4.3 Risk Categorization

Categorization of participants into high, intermediate and low risk categories for both groups was done based on the ranges in outcome variables as provided in Table 4. This was done in order to observe the shift clinical risk categories as the treatment proceeded.

4.4 Adherence to Protocol Evaluation

At day 30 (visit 2) and day 90 (visit 3) subjects in both groups were questioned about their adherence to protocol via a questionnaire (Appendix B). Subject’s responses regarding frequency of oral rinsing, tooth brushing and flossing were recorded.

4.5 Statistical Analysis

Initial exploration using histograms and normal quantile diagnostic plots showed that counts of MS and LB at each visit were strongly skewed towards higher values. Zero values for LB were substituted with a “1” value prior to computation of base 10 logarithm transformations. However due to extreme values, log_{10} transformation failed to achieve approximate normality in distribution of these variables. The small sample size meant that the data were not robust to violations of assumptions of independence, normality and homogeneous variances. Consequently while means, standard deviations,
and range for clinical endpoints were reported in descriptive tables and figures, differences were tested for statistical significance using two-sample Wilcoxon rank-sum (Mann-Whitney) test; a nonparametric analogue of the independent samples t-test. Analytic analysis began with a baseline comparison of patient characteristics in intervention and control groups. Change from baseline for clinical endpoints was calculated by subtracting the current parameter from its baseline value. The strength and direction of the relationship between ATP and MS counts was tested using Spearman's rank-order correlation. The randomization schedule and statistical analyses of data were performed using Stata 12.0 statistical software (StataCorp LP College Station, TX).
5 RESULTS

The results of the study are focused on two aims. The primary aim was to examine the effectiveness of the CariFree Treatment protocol in reducing caries risk. The outcome variables used to measure effectiveness of protocol were:

i. Plaque ATP bioluminescence scores read in Relative Light Units (RLU) ranging from 0-9999. Any score 1500 or below is considered low risk.

ii. Salivary Buffering capacity read as a laboratory pH measurement. pH measurements obtained were ranging from 3.0-6.1 for the entire study population.

iii. Salivary Mutans Streptococcus (MS) counts

iv. Salivary Lactobacillus (LB) counts

The secondary outcome of interest was the correlation between the chairside ATP bioluminescence scores and MS counts.

Table 5 describes the age and characteristics of subjects enrolled in this study, including the randomized allocation of intervention and control groups. The age of the participants ranged from 22-58 years. The male:female ratio was 5:7 in the intervention and 4:8 in the control group. All the outcome variables pre-randomization had no statistically significant difference between the two groups. Plaque and saliva samples were obtained at baseline, 1 month and 3 months. Only one subject (from the control group) was lost at visit 3.
Tables 6 and 8 describe the clinical end points of the outcome variables at end of 1 month and 3 month respectively while Tables 7 and 9 describe the difference in the clinical end points at 1 month and 3 months from baseline respectively. The primary end point of ATP bioluminescence appears to have a larger variation from baseline in the intervention group than control group at both time periods. This change can also be appreciated in Figure 7. The mean values in both intervention and control group show a tendency to decrease at the 1 month time although both do not reach statistical significance.

While mean values for buffering capacity remained unaltered in the control group between baseline and three-month follow-up, the corresponding values for the intervention group showed a tendency to increase over time (Figure 8). However these differences failed to reach statistical significance.

Figures 9 and 10 show the mean MS and LB counts values respectively at baseline, 1 month and 3 months for both groups. The mean values of MS and LB in the control group remained unaltered during the study, while those for the intervention group showed a tendency to increase from baseline at the 1 month measurement, and then appeared to decrease resulting in a negative change from baseline at the 3 month (Table 9).

Categorization of participants into high, intermediate and low risk categories for both groups was further done based on the ranges in outcome variables as provided in Table 4. In the control group a reduction was observed in the number of subjects in the high risk category based on MS counts from 8 to 3 (62.5%) subjects from baseline to visit 3 and reduction by 60% in the buffering capacity (Figure 11). In the intervention group
the total number of subjects in the high risk category based on ATP scores reduced by 66.6% (4 out of 6) at visit 3 from baseline (Figure 12). A similar reduction in the high risk category of 42.8% in SM counts and 33.3% for buffering capacity from baseline to Visit 3 was observed. No remarkable change based on LB counts was observed.

The adherence to protocol questionnaire (Appendix B) showed that at visit 2 seven of the twelve subjects (58.3%) randomly assigned to the intervention group complied to brushing twice daily. This was a slight improvement from the visit 1 compliance of 50%. Compliance to rinsing two times daily also improved from 41.6% at visit 1 to 66.6 % at visit 2. All of the non-compliant subjects brushed and rinsed at least once daily. Flossing once daily had adherence rates of 83.3% at visit 1 and 100% at visit 2.

For the subjects assigned to the control group, compliance rates for brushing twice daily started at 91% at visit 1 but dropped to 63.6% at visit 2. Daily compliance for flossing dropped similarly from 75% to 46% at visit 2. No data was recorded for rinsing, since that was not part of protocol for the control group.

The correlation between the chairside ATP bioluminescence test and the laboratory based MS count was determined statistically (Table 11) using the Spearman’s rank-order correlation tests. At all evaluation times the correlation was poor.
DISCUSSION

The results of this study show no significant difference between intervention and control groups at 3 months post initiation of treatment based on the study outcomes. The study outcomes under investigation were ATP bioluminescence scores, buffering capacity, Mutans Streptococci and Lactobacillus counts.

The intervention group received CariFree treatment and maintenance mouthrinse, neutralizing gel, boost oral spray and samples of xylitol gum. The treatment rinse contains sodium fluoride 0.05%, water, xylitol, sodium hydroxide and sodium hypochlorite. The amount of xylitol delivered per dose with this rinse is approximately 1gm (x 2 times daily). Efficacy of xylitol is dose-dependent, and the minimum amount needed to provide a beneficial effect on the plaque biofilm has been shown to be 5-6 grams/day, divided into three to four doses, for 5-10 minutes per exposure [70]. Hence the xylitol from the rinse by itself is insufficient to show therapeutic effect. It could be supplemented by oral xylitol mints/chewing gums after reviewing the amount of xylitol being delivered through them, since the effects of xylitol on MS plateau between 6.44 g and 10.32 g. xylitol/day [71].

The combination of agents present in the rinse however might have worked in the initial phase to provide a “shock” treatment to the overall bacterial load present in the biofilm. This is reflective in the trend observed at visit 1 (1 month) post treatment in the
ATP bioluminescence scores. The MS and LB scores appear to follow an opposite trend to the ATP scores. This may be explained by the following sequence of events. Sodium hypochlorite and its decomposition byproducts such as hypochlorous acid, by nature are highly reactive. They combine with the first surface they contact and lose their potency rapidly. Considering the inherent protective structure of the biofilm and reduced access to antimicrobials [72], there is a possibility that only the outer layers of the biofilm were penetrated by the rinse. This would then facilitate the MS and LB that were buried deeper in the tiers to become exposed and more susceptible to collection via the stimulated saliva collection technique, causing the apparent trend observed at visit 1. No such trend was observed in the control group with the bacterial counts. A small trend towards decline in the ATP scores was observed but that might be attributed to an improvement in oral hygiene measures in both groups since enrollment in the study.

At visit 2 (3 months), measurements were made after using the maintenance rinse for 60 days. The maintenance rinse contains sodium fluoride 0.05%, water, xylitol and sodium hydroxide. The general trend observed for both ATP scores and bacterial counts was a reduction in total number in the intervention group suggestive of possible efficacy of treatment; however the difference observed did not assume statistical significance. A similar trend but to a lesser degree was observed in the control group.

The buffering capacity of saliva is a measure of the ability to neutralize acid. As observed, the buffering capacity seems to have an upward trend as the compliance of the patient’s improved (Table 10). There is good evidence that behavior modification and counseling can improve adherence to protocol and shift the trends of study outcomes in health care settings [73]. A coaching method adopted by the health care provider places
them in a position to support and motivate behavior change. During the counseling sessions a possible confounding factor came to light and was associated with the poor compliance to rinsing in the first month due to the unpleasant taste of the treatment rinse.

The Rapid ATP Bioluminescence Assays using luciferase enzymes as used in our study have been found to have a high correlation with the total plaque mass [10, 11, 67, 74]. A study looking at the assay of the plaque and saliva samples around orthodontic brackets in 14 individuals with ages 11-17 and concluded that ATP-driven bioluminescence is highly predictive of the numbers of total oral bacteria and total oral streptococci, and by statistical extension, also reflective of the numbers of mutans streptococci [11]. The same core group further evaluated the correlation between mutans streptococci and ATP Bioluminescence for 33 subjects 7-12 years in age [69]. In this study the authors took plaque samples from the mixed dentition at 4 different sites- facial of right maxillary first molar, maxillary left central incisor and lingual of left mandibular premolar and anterior incisor followed by collection of a stimulated saliva specimen. Pearson correlation coefficients of 0.682, 0.611, and 0.548 were identified for total oral bacteria, total oral streptococci, and MS, respectively. In our study we were looking specifically at the correlation between the total MS counts in saliva and ATP Bioluminescence of the plaque mass. Contrary to the findings of the previously mentioned study we found a weak to poor correlation between the two. There could be a number of reasons to explain this difference. Firstly, as observed by the group in their discussion of their study results, a stronger correlation was achieved when using the increased statistical power of the larger sample number contained in the composite plaque and saliva specimen set. Secondly, the plaque readings in our specimen set were made
from only one site as per the manufacturer instructions (the mandibular incisal lingual surface) as opposed to different areas around the mouth. Thirdly, no correlation was established in this study with the total bacterial counts, which is also a limitation of our study.

While the outcomes have not shown any statistically significant difference between the two groups or between the starting and end point of treatment, there is a shift in the individual risk categories of the 24 patients observed through the course of the treatment suggestive that perhaps the time frame of this study was insufficient to determine the efficacy of treatment statistically.

Due to limitations in time and cost, the total number of subjects in each group was 12, out of which 1 was lost to follow up in the control group at the third visit. While the total sample population was randomized using stratified block randomization, all subjects were high caries risk and displayed a wide range in their ATP and bacterial count scores, reducing the power of the sample. All attempts were made to motivate the subjects to improve their oral hygiene and remain adherent to the protocol but making an objective assessment such as recording plaque indices at each visit would have provided useful information. Additionally, as a prelude to the 3 month treatment, a run-in phase, to monitor the compliance of the subjects might have helped eliminate non-compliant subjects. As opposed to some previous studies, the total bacterial counts were not calculated as a measure of the effectiveness of the CariFree treatment rinses; only the cariogenic species MS and LB were monitored over a period of 3 months [11, 69]. Lastly, if this study could be extended to follow the progress of these subjects for a longer duration, a statistically significant difference might be appreciated.
Within the limitations of this study the following conclusions were drawn:

1. The CariFree home care protocol did not statistically reduce the caries risk in “high” caries risk adults after a 90 day regimen when compared with traditional oral hygiene practices.

2. There was poor agreement between CariScreen ATP bioluminescence assay and laboratory streptococcal counts, thereby implying that the reduction in overall plaque mass did not correlate with the reduction in percentage MS. The CariScreen chair side measurement tool though may prove to be a usual adjunct in patient motivation and monitoring progress in plaque control.

3. While a statistically significant reduction in caries risk was not obtained, there was a noticeable shift in the number of subjects in the intervention group from a higher risk category to a lesser risk category in all outcomes evaluated except LB counts. A similar trend, but to a lesser degree was noticed for the control group.

4. The adherence to protocol was less than 70% in both groups but a trend was noticed towards improvement in buffering capacity with improvement in compliance.
Fig. 1. Schematic simulating the behavior of similar bacterial cultures containing MS and LB, on addition of sugar and (a) controlling pH at 7.0 and (b) allowing pH to fall for 6 hours.
Fig. 2. Adapted schematic of the balance between progressive dental disease at one end and protective factors maintaining a low risk [7].

Fig. 3. Schematic of chemical structure (2R,3r,4S)-Pentane-1,2,3,4,5-pentol (Xylitol)
Screening and enrollment

High caries risk
CRA form, ATP scores

Baseline assessment
(ATP bioluminescence score, saliva collection)

Treatment group (n=12)
- *CariFree Treatment rinse* used 2 times daily for 30 days
- *CariFree Maintenance rinse* Used twice daily after brushing and flossing from day 30 till day 90
- *CariFree Oral neutralizer gel* used twice daily as a toothpaste supplement for 90 days
- *CariFree Boost* used 2-3 sprays in mouth as often as needed to relieve dry mouth and neutralize acids. Between meals and before bedtime recommended (90 days)
- *CariFree Xylitol gum* chewed 2 pieces, 3-5 times daily. Recommended after meals or when dry mouth/bad breath occurs (90 days).

Control group (n=12)
- Continue regular standard of care oral hygiene measures

Block randomization
1 Block=4 subjects

visit 2 (30 day) assessment
(ATP bioluminescence scores, saliva collection)

visit 3 (90 day) assessment
(ATP bioluminescence scores, saliva collection)

Fig. 4. Flowchart depicting the Study Design
Fig. 5. Plaque sample collection with CariScreen swabs from lingual surface of mandibular anterior teeth (a and b).
Fig. 6. Spiral PlaterTM (a) used for spiral plating process (b) over a selective agar media (c) for 24h and bacterial counts done by zones (d).
Fig. 7. Mean (s.e.) ATP bioluminescence scores for intervention and control groups at baseline, and at one month and three months post-randomization.

Fig. 8. Mean (s.e.) buffering capacity for intervention and control groups at baseline, and at one month and three months post-randomization.
Fig. 9. Mean (s.e.) Mutans streptococci (x 10^3) for intervention and control groups at baseline, and at one month and three months post-randomization.
Fig. 10. Mean (s.e.) Lactobacillus ($10^3$) for intervention and control groups at baseline, and at one month and three months post-randomization
Fig. 11. Percentage (y axis) and number (table) of subjects in control group classified in high, intermediate and low risk categories (Table 4) at visits 1, 2, and 3 based on (a) ATP bioluminescence scores, (b) buffering capacity, (c) Streptococcus Mutans counts and (d) Lactobacillus counts.
Fig. 12. Percentage (y axis) and number (table) of subjects in intervention group classified in high, intermediate and low risk categories (Table 4) at visits 1, 2, and 3 based on (a) ATP bioluminescence scores, (b) buffering capacity, (c) Streptococcus Mutans counts and (d) Lactobacillus counts.
TABLES

Table 1. Risk factors based on clinical examination

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>(1)</td>
<td>Intermediate or High <em>Streptococcus</em> Mutans and <em>Lactobacillus</em> counts</td>
</tr>
<tr>
<td>(2)</td>
<td>Inadequate saliva flow (&lt;0.07 ml/min stimulated)</td>
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<tr>
<td>(3)</td>
<td>Large number of filled teeth</td>
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<tr>
<td>(4)</td>
<td>Visible heavy plaque</td>
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<tr>
<td>(5)</td>
<td>Recession with exposed</td>
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<tr>
<td>(6)</td>
<td>Defective restorations with open margins</td>
</tr>
<tr>
<td>(7)</td>
<td>Fixed/Removable dental protheses</td>
</tr>
<tr>
<td>(8)</td>
<td>Deep pits and fissures</td>
</tr>
<tr>
<td>(9)</td>
<td>Noncavitated Lesions</td>
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Table 2. Risk factors based on history

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1</td>
<td>Existing or recent history of caries</td>
</tr>
<tr>
<td>2</td>
<td>No dental visit in the past 6 months</td>
</tr>
<tr>
<td>3</td>
<td>Limited lifetime exposure to water fluoridation</td>
</tr>
<tr>
<td>4</td>
<td>Brushes once a day or less</td>
</tr>
<tr>
<td>5</td>
<td>Salivary reducing factors (medication, radiation)</td>
</tr>
<tr>
<td>6</td>
<td>Frequent snacking between meals (&gt;3 times)</td>
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<tr>
<td>7</td>
<td>Health behavior risks (smoking, eating disorders)</td>
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<td>8</td>
<td>Low socio-economic status</td>
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<tr>
<td>9</td>
<td>Low education levels</td>
</tr>
<tr>
<td>10</td>
<td>Impaired cognitive ability</td>
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Table 3. Example of pre-prepared envelopes for randomization of subjects

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<tr>
<th>Envelope 1</th>
<th>Envelope 2</th>
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<th>Envelope 4</th>
<th>Envelope 5</th>
<th>Envelope 6</th>
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<tbody>
<tr>
<td>Intervention</td>
<td>Intervention</td>
<td>Control</td>
<td>Intervention</td>
<td>Control</td>
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Table 4. Risk categories based on accepted values in literature and industry for ATP bioluminescence, buffering capacity, Mutans Streptococcus and Lactobacillus counts

<table>
<thead>
<tr>
<th></th>
<th>Low Risk</th>
<th>Intermediate Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (RLU)</td>
<td>0-1500</td>
<td>1501-4500</td>
<td>4500-9999</td>
</tr>
<tr>
<td>Buffering capacity- final pH (diluted 1 to 4 in 0.005N HCl):</td>
<td>5.0-7.0</td>
<td>4.1-4.9</td>
<td>≤4.0</td>
</tr>
<tr>
<td>Mutans streptococci (MSB counts)</td>
<td>&lt; $10^3$ CFU/ml</td>
<td>2$x10^3$ to 9$x10^4$ CFU/ml</td>
<td>1$x10^5$ or greater CFU/ml</td>
</tr>
<tr>
<td>Lactobacillus (Ragosa SL)</td>
<td>&lt; $10^3$ CFU/ml</td>
<td>1$x10^3$ to 9$x10^3$ CFU/ml</td>
<td>1$x10^6$ or greater CFU/ml</td>
</tr>
</tbody>
</table>
Table 5. Age, clinical and salivary characteristics of eligible patients in intervention and control groups at enrollment

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total sample N=24</th>
<th>Intervention group n=12</th>
<th>Control group n=12</th>
<th>P-value(^{(a)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Dev.</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.5</td>
<td>10.1</td>
<td>22.0</td>
<td>58.0</td>
</tr>
<tr>
<td>Buffering capacity final pH</td>
<td>4.5</td>
<td>1.2</td>
<td>3.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Chair-side pH</td>
<td>6.5</td>
<td>0.4</td>
<td>5.5</td>
<td>7.0</td>
</tr>
<tr>
<td>ATP (RLU)</td>
<td>4613.8</td>
<td>2770.5</td>
<td>1638.0</td>
<td>9972.0</td>
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<tr>
<td>Mutans streptococci (MSB) (x 10(^{3}))</td>
<td>517.4</td>
<td>975.5</td>
<td>0.5</td>
<td>4600.0</td>
</tr>
<tr>
<td>Lactobacillus (Ragosa SL) (x 10(^{3}))</td>
<td>485.7</td>
<td>1735.1</td>
<td>0.0</td>
<td>8600.0</td>
</tr>
<tr>
<td>Salivary flow rate (mL/minute)</td>
<td>1.4</td>
<td>1.1</td>
<td>0.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

\(^{(a)}\) P-values test null hypothesis of equivalence between intervention and control groups using two-sample Wilcoxon rank-sum (Mann-Whitney) test
Table 6. Clinical endpoints for subjects in intervention and control groups at baseline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intervention group n=12</th>
<th>Control group n=12</th>
<th>P-value(^{(a)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Dev.</td>
<td>Min</td>
</tr>
<tr>
<td>Buffering capacity final pH</td>
<td>4.1</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Chair-side pH</td>
<td>6.6</td>
<td>0.4</td>
<td>5.8</td>
</tr>
<tr>
<td>ATP (RLU)</td>
<td>2902.8</td>
<td>1931.8</td>
<td>467.0</td>
</tr>
<tr>
<td>Mutans streptococci (MSB) (x 10^3)</td>
<td>1211.3</td>
<td>2711.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Lactobacillus (Ragosa SL) (x 10^3)</td>
<td>2277.8</td>
<td>7160.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Salivary flow rate (ml/minute)</td>
<td>1.3</td>
<td>0.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

\(^{(a)}\) P-values test null hypothesis of equivalence between intervention and control groups using two-sample Wilcoxon rank-sum (Mann-Whitney) test
Table 7. Change (difference from baseline)\(^{(a)}\) in primary endpoint and other clinical endpoints, one month post-randomization

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intervention group (n=12)</th>
<th>Control group (n=12)</th>
<th>(P)-value(^{(b)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean difference(^{(b)})</td>
<td>Std. Dev.</td>
<td>Mean difference(^{(b)})</td>
</tr>
<tr>
<td>Buffering capacity final pH</td>
<td>-0.15</td>
<td>1.48</td>
<td>-0.34</td>
</tr>
<tr>
<td>Chair-side pH</td>
<td>0.10</td>
<td>0.34</td>
<td>-0.15</td>
</tr>
<tr>
<td>ATP (RLU)</td>
<td>-1921.92</td>
<td>2275.04</td>
<td>-913.75</td>
</tr>
<tr>
<td>Mutans streptococci (MSB) (x 10^3)</td>
<td>542.66</td>
<td>3177.34</td>
<td>26.93</td>
</tr>
<tr>
<td>Lactobacillus (Ragosa SL) (x 10^5)</td>
<td>1424.65</td>
<td>7721.89</td>
<td>-28.42</td>
</tr>
<tr>
<td>Salivary flow rate (ml/minute)</td>
<td>-0.19</td>
<td>1.17</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Change was computed by subtracting the one month value from the baseline value; positive numbers indicate an increase from baseline.

\(^{(b)}\) \(P\)-values test null hypothesis of equivalence between intervention and control groups using two-sample Wilcoxon rank-sum (Mann-Whitney) test.
Table 8. Clinical endpoints for patients in intervention and control groups three months post randomization

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intervention group n=12</th>
<th>Control group n=12</th>
<th>P-value(^{(a)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Dev.</td>
<td>Min</td>
</tr>
<tr>
<td>Buffering capacity final pH</td>
<td>5.0</td>
<td>1.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Chair-side pH</td>
<td>6.5</td>
<td>0.5</td>
<td>5.5</td>
</tr>
<tr>
<td>ATP (RLU)</td>
<td>2984.6</td>
<td>1655.1</td>
<td>780.0</td>
</tr>
<tr>
<td>Mutans streptococci (MSB) (x 10^3)</td>
<td>237.5</td>
<td>395.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Lactobacillus (Ragosa SL) (x 10^3)</td>
<td>170.6</td>
<td>366.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Salivary flow rate (ml/minute)</td>
<td>1.8</td>
<td>1.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^{(a)}\) P-values test null hypothesis of equivalence between intervention and control groups using two-sample Wilcoxon rank-sum (Mann-Whitney) test
Table 9. Change (difference from baseline) \(^{(a)}\) in primary endpoint and other clinical endpoints, three months post-randomization

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intervention group n=12</th>
<th>Control group n=11</th>
<th>(P)-value(^{(b)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean difference(^{(b)})</td>
<td>Std. Dev.</td>
<td>Mean difference(^{(b)})</td>
</tr>
<tr>
<td>Buffering capacity final pH</td>
<td>0.80</td>
<td>2.28</td>
<td>0.06</td>
</tr>
<tr>
<td>Chair-side pH</td>
<td>0.04</td>
<td>0.54</td>
<td>-0.09</td>
</tr>
<tr>
<td>ATP (RLU)</td>
<td>-1840.08</td>
<td>2263.85</td>
<td>-1161.36</td>
</tr>
<tr>
<td>Mutans streptococci (MSB) ((x \times 10^3))</td>
<td>-431.11</td>
<td>1081.82</td>
<td>-83.63</td>
</tr>
<tr>
<td>Lactobacillus (Ragosa SL) ((x \times 10^3))</td>
<td>-682.50</td>
<td>2492.20</td>
<td>-81.18</td>
</tr>
<tr>
<td>Salivary flow rate (ml/minute)</td>
<td>0.28</td>
<td>1.51</td>
<td>0.16</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Change was computed by subtracting the three month value from the baseline value; positive numbers indicate an increase from baseline.

\(^{(b)}\) \(P\)-values test null hypothesis of equivalence between intervention and control groups using two-sample Wilcoxon rank-sum (Mann-Whitney) test
Table 10.  Mean (95% confidence interval [CI]) change in buffering capacity\(^{(a)}\) at three months from baseline, according to compliance with home care regime, intervention group (n=12) \(^{(b)}\)

<table>
<thead>
<tr>
<th>Sum of compliant behaviors</th>
<th>N subjects</th>
<th>Mean change in buffering capacity</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>-0.80</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0.47</td>
<td>-0.61, 1.55</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1.77</td>
<td>-2.24, 5.79</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Change was computed by subtracting the three month value from the baseline value; positive numbers indicate an increase from baseline.

\(^{(b)}\) Compliant behaviors were: tooth brushing 2x daily; CariFree-rinse 2x daily, use of dental floss 1x daily.
Table 11. Correlation\(^{(a)}\) of chairside ATP bioluminescence test (CariScreen) and salivary Streptococcus mutans count (CFU/mL of saliva) test at three time points in two treatment groups

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment group</th>
<th>Rho</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Intervention</td>
<td>0.105</td>
<td>0.746</td>
</tr>
<tr>
<td>Baseline</td>
<td>Control</td>
<td>-0.559</td>
<td>0.059</td>
</tr>
<tr>
<td>One month</td>
<td>Intervention</td>
<td>-0.406</td>
<td>0.190</td>
</tr>
<tr>
<td>One month</td>
<td>Control</td>
<td>-0.098</td>
<td>0.762</td>
</tr>
<tr>
<td>Three months</td>
<td>Intervention</td>
<td>-0.406</td>
<td>0.190</td>
</tr>
<tr>
<td>Three months</td>
<td>Control</td>
<td>-0.098</td>
<td>0.762</td>
</tr>
</tbody>
</table>

(a) Spearman's rank-order correlation tests the null hypothesis that the ranks of one variable do not covary with the ranks of the other variable.
APPENDICES

Appendix A

Caries Risk Assessment

Adults/Children Age 6 and Over

Patient Name: ___________________________ Date: ________________

Instructions: Check all answers that apply.

If 1 or more Disease Indicators or 2 or more Risk Factors are circled, then this patient is at risk and therapeutic intervention is recommended.

1. **ASSESS**
   **DISEASE INDICATORS**

<table>
<thead>
<tr>
<th>Disease Indicator</th>
<th>AT RISK</th>
<th>LOW RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible Cavitations</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Radiographic Lesions</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>White Spot Lesions</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Cavity in Last 3 Years</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

2. **RISK FACTORS**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>AT RISK</th>
<th>LOW RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible Plaque</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Inadequate Saliva Flow</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Hyposalivary Medications</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Acidic Beverages</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Frequent Snacking (1-3 times daily)</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Appliances Present</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Deep Pits and Fissures</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Other</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

3. **TESTING**

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CariScreen</td>
<td>9.999 - 1,501</td>
</tr>
</tbody>
</table>

2. **DIAGNOSE**

Risk Assessment: AT RISK

3. **PRESCRIBE**

☐ Treatment Kit

☐ Maintenance Kit

I understand my risk for caries based on this assessment, as well as the benefits of the recommendations for therapeutic intervention.

Release Signature: ___________________________

---

* Based on clinically proven Caries Risk Assessment Form in the Featherstone 2003-2005 study.
* Caries risk criteria as defined by the American Dental Association Council on Scientific Affairs, JADA August 2006.
Appendix B

Adherence to Protocol Questionnaire

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>How often do you brush your teeth in a day</td>
<td>Not at all, Once, Twice or more</td>
</tr>
<tr>
<td>How often do you rinse with CariFree mouth rinse in a day</td>
<td>Not at all, Once, Twice or more</td>
</tr>
<tr>
<td>How often do you floss your teeth in a day</td>
<td>Not at all, Once or more</td>
</tr>
</tbody>
</table>
9 REFERENCES


