Effect of Remineralizing Agents on Dental Erosion In a Reduced Salivary Flow Population: An In Situ Model

BY

ADRIANA SEMPRUM-CLAVIER
D.D.S., Universidad Central de Venezuela, Caracas 1996
D.D.S., University of Detroit Mercy, Detroit, MI 2004

THESIS

Submitted as partial fulfillment of the requirements
for the degree of Master of Clinical and Translational Sciences
in the Graduate College of the
University of Illinois at Chicago, 2018

Chicago, Illinois

Defense Committee:

Jack Zwanziger, Chair and Advisor

Ana Bedran-Russo, Advisor, Restorative Dentistry

Lyndon Cooper, Oral Biology

Grace Viana, Orthodontics

ACKNOWLEDGEMENTS

I would like to express my most sincere thanks and appreciation for the members of my thesis committee, Dr. Jack Zwanziger, Dr. Lyndon Cooper, for their support and guidance, Dr. Grace Viana, for her guidance, willingness to answer questions analyzing and helping me complete this thesis. Lastly, to Dr. Ana Bedran–Russo for her mentorship, patience, and unlimited amount of time spent to help and support my efforts in research and in the completion of this Masters Degree. To Dr. Ben Amaechi, for sharing his knowledge and time regarding his research model.

Thank you to my friends and coworkers Priscilla Chang, Courtney Lamb, Alexandra Rodriguez and Daisy Salazar for their friendship, support, time and patience with the completion of this project.

Finally I would like to thank my family for their unlimited love and support throughout our journey together.

ASC

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADA	American Dental Association
CI	Confidence interval
CPP ACP	Casein Phosphopeptide-Amorphous calcium phospate
F	Fluoride
GERD	Gastroesophageal reflux disease
IRB	Institutional Review Board
MFP	Monofluorophosphate
NaF	Sodium Fluoride
PPM	Part per million
SD	Standard Deviation
SMH	Surface Microhardness Test
SMH1	Pre-treatment surface microhardness Test
SMH2	Post-treatment surface microhardness Test
SSF	Sjögren's Syndrome Foundation

SUMMARY

The prevalence of dental erosion is increasing. Several extrinsic factors can affect erosive lesion development such as diet, tooth brushing, and fluoride exposure. Acidic diets can lead to the loss and softening of tooth structure, and as a consequence, tooth surfaces are more prone to wear and abrasion. Intrinsic factors for erosion include the protective properties of saliva, pellicle formation, unstimulated salivary flow rate, and buffering capacity. Many *in vitro* and *in vivo* studies have shown that saliva assists with the remineralization process in the presence of fluoride, phosphate and calcium ions. However, when saliva flow is not ideal, patients present high risk for dental caries and dental erosion due to lack of remineralizing ions present and the buffering capacity.

There are several remineralizing agents available to prevent and reverse early stages of dental erosion. Fluoride has been reported by numerous studies for its ability to remineralize softened erosive surface lesions. It has been suggested by previous studies that highly concentrated fluoride solution increases the remineralization of caries and dental erosion lesions. Very little is known about the efficacy of remineralizing agents in patients with impaired salivary flow.

MI Paste is another topical remineralizing agent, which provides available calcium and phosphates to prevent and reverse caries, dental erosion and tooth hypersensitivity. MI Paste consists of a casein phosphopeptide amorphous calcium phosphate compound CPP-ACP technology that helps to stabilize

calcium and phosphates in the oral environment and delivers ions to the tooth surface.

The purpose of this *in situ* crossover design study is to compare the remineralization efficacy of a high concentration fluoride dentifrice and a remineralizing agent used by a patient, with reduced salivary flow after one week of home application period; specifically Prevident 5000 Toothpaste (1.1 %NaF), and MI Paste (CPP-ACP).

The aims of this study are to (1) analyze the efficacy of two remineralizing agents on erosive surface lesion after one-week application period; (2) compare the remineralization effectiveness of each agent on reduced salivary flow patients and optimal salivary flow patients.

This research may lead to an increased understanding of the dynamic of remineralization after an erosive insult to tooth structure when salivary flow is not optimal. Understanding the efficacy of these two products on remineralization in patients with reduced salivary flow may be helpful to provide future guidelines for patients who are at higher risk of developing dental erosion.

1. INTRODUCTION

1.1 Background

The prevalence of dental erosion is rising worldwide in all ages (Hamachi, 2005). This is evident from different prevalence studies conducted in the United Kingdom and the US showing higher percentage of individuals affected by erosion in different age groups (Amaechi, 2005). Dental erosion consists of loss of surface tissue, as a consequence of exposure to a variety of acids of non-bacterial origin from intrinsic or extrinsic sources (Lussi, 2004, 2006). As teeth are exposed to acids, the pH of the mouth drops below 5.5 leading to dissolution of the hydroxyapatite, making teeth softer and more susceptible to mechanical wear.

Saliva can provide protection from dental erosion through its buffering and remineralizing capacities. In addition, studies have shown that saliva at its optimal flow, and supersaturated with calcium, phosphate and fluoride may have not only a protective effect but also a reparative effect on early enamel erosion (Hall et al., 1999).

Unfortunately, low salivary flow rate not only compromises the ability to remineralize erosive lesions but also can increase the risk of dental erosion. Specifically, decreased unstimulated salivary flow can lead to a lower pH level in the mouth causing enamel demineralization and compromising the possible reparative effect on eroded lesions (Hall et al., 1999).

The prevalence of patients with salivary gland hypofunction or hyposalivation has increased steadily over the last decade for a variety of reasons (Navazesh, 2008). The condition is not only more common with increasing age but can also be caused by medications such as: anti-hypertensives, anti-depressants, diuretics, anti-allergics, substance abuse, therapeutic radiation, autoimmune diseases such as Sjögrens disease and other chronic conditions (Billings, 1993; Pianprach, 2009; Tan, 2017). Hyposalivation is the condition of having reduced saliva production and is best diagnosed by measuring salivary flow rate. An unstimulated salivary flow rate below 0.2ml/minute indicates hyposalivation (Hara et al., 2005).

Saliva plays a crucial role in the dynamic process of demineralization and remineralization providing ions of calcium, phosphates and fluoride, supersaturating the oral environment to always promote remineralization. Several reports (Buzalaf et al., 2011; Hanig, 2014; Vukosavlevic et al., 2014) discuss the critical role of salivary factors on dental erosion. The protective function of saliva is attributed to several reasons such as: the formation of the acquired pellicle, its diluent action, clearance ability eliminating acids through swallowing and chewing, its buffering capacity and state of supersaturation with respect to the tooth, providing calcium, phosphate and fluoride necessary for remineralization. While the composition of saliva on the protection against dental erosion is important, the salivary flow rate significantly impacts patients raising their risk to develop erosion (Jarvenin, 1991).

When salivary flow is not optimal, the oral environment suffers an imbalance where loss of minerals from tooth structure leads to enamel breakdown. Therefore, it is critical to understand the role of saliva in enamel remineralization in patients with chronic hyposalivation and to assess the effectiveness of recommended early intervention to this population at risk before dental erosion develops. Hall et al. (1999) compared the protective effect of saliva on dental erosion *in vitro and in situ* showing a highly significant difference in the protection from saliva *in situ* compared to an *in vitro* protocol. This greater protection effect from saliva can be attributed to the presence of organic layers covering the specimen, the presence of fluoride in situ as well as the quantity and chemical composition of saliva *in situ as compared to in vitro* experiments.

Many adjunct topical agents have been developed to assist saliva in the remineralization process such as fluoride and calcium-based products among others. Its effectiveness has been widely demonstrated in patients that present optimal salivary flow, which enhances the ability of these products to combat dental erosion. These products have not been proven effective in individuals with chronic hyposalivation, which can compromise the reparative effect of these products. However, these products are commonly recommended to patients with reduced salivary flow to enhance remineralization. The lack of normal salivary flow might compromise its effectiveness.

In this study the population of interest are individuals who suffer from hyposalivation or dry mouth. The prevalence of hyposalivation is difficult to determine as it varies by geographical zone and age ranging from 6% to 46%

increasing with age (Niklander et al., 2017) Patients suffering from hyposalivation lack the benefits provided by saliva in the repair and remineralization process. Therefore, these patients are considered to be at extreme risk for dental caries and dental erosion, and need timely preventive interventions before demineralization occurs. Moreover, these individuals might not benefit from similar recommendations for patients with optimal saliva flow. Moreover, most studies, testing the role of saliva have been performed *in vitro*, or have only included healthy individuals with optimal salivary flow showing effectiveness with products such as fluoride and calcium phosphates. Thus, effective clinical recommendations are necessary to prevent enamel demineralization in this population.

1.2 **Purpose of Study**

The purpose of the study was to compare *in situ* the enamel remineralization effectiveness of a high concentration fluoride dentifrice and a calcium-based remineralizing agent used at home by healthy patients and patients with reduced salivary flow after a one week home application period; specifically a high-fluoridated dentifrice, Prevident 5000 (Colgate-Palmolive Co., New York City, NY, USA), and a complex of casein phosphopeptide amorphous calcium phosphate CPP-ACP, MI Paste™ (GC America, Alsip, Illinois, USA).

1.3 Specific Aims

Specific Aim 1: To compare the difference in enamel remineralization response between the reduced salivary flow patients and normal salivary flow patients for each remineralizing agent.

Specific aim 2: To compare the difference in remineralization response after an erosive challenge (SMH1) and post-intervention (SMH2) after one-week application of two remineralizing dentifrices: a fluoride based agent and a calcium-based agent.

1.4 Hypotheses

Null Hypothesis 1**:** There will be no mean difference in percent surface microhardness recovery between normal salivary flow patients and reduced salivary flow patients, regardless of the remineralization strategy.

Null Hypothesis 2: There will be no mean difference between Pre-test (SMH1) and Post-test (SMH2) surface enamel microhardness for each remineralizing agent.

2. REVIEW OF LITERATURE

2.1 Prevention of Dental Erosion

The prevalence of dental erosion is rising for a variety of reasons (Lussi et al., 2009). Elderly patients are retaining their natural teeth and are at risk of dental erosion due to increased occurrence of hyposalivation, poor oral health, chronic health conditions, and neglection of dental care.

Moreover, younger patients are presenting more pathological tooth wear due to acidic diet and endogenous acids from acid reflux or eating disorders, leading to loss and softening of tooth structure (Lussi et al., 2004). According to Tahmasebi (2006), in the developed world, over 50% of liquids consumed are sodas and fruit based drinks. The commercialization of the soft drinks has worsened the problem showing an increase on dental erosion in every demographic (Tahmassebi, 2006). A systematic review (Salas et al, 2015) evaluating the prevalence of teeth wear in children and adolescents reported a range between 7.2% and 95%. This high variability can be attributed to the different diagnostic indices used for dental erosion, sample size, as well as the geographic location. Middle East and Africa showed the highest prevalence of 41.4% compared to America and Asia of 20%.

According to Jarvinen (1991), patients with reduced salivary flow rate are five times at risk of erosion than those with normal flow rate. Along with an increase in prevalence, also the severity of dental erosion is rising due to factors such as increased individual susceptibility, changes in behavior, consumption of more

acidic food and beverages, increased prevalence of eating disorders and the absence of protective mechanism. Dental erosion consists of permanent loss of surface tissue of non-bacterial origin, as a consequence of exposure to a variety of acids from extrinsic or intrinsic sources. Extrinsic factors involve the consumption of acidic food and beverages and industrial fumes. Possible intrinsic factors comprise frequent regurgitation and GERD. Its multifactorial nature helps to explain its severity. The severity of dental erosion depends on many factors such as the erosive potential of the agent and its contact time with the hard tissues, the individual susceptibility, the soft tissue anatomy and movement, behavioral aspects, as well as the rate of secretion of saliva and its composition. Intrinsic factors related to saliva like pellicle formation, unstimulated salivary flow rate and buffering capacity could affect the individual's susceptibility to dental erosion (Hara et al; 2005, Schlueter, 2014).

2.2 **The Role of Saliva on Dental Erosion**

Studies have shown that saliva at an optimal flow supersaturated with its calcium, phosphate and fluoride content may have a reparative effect on early enamel erosion (Amaechi and Hingham, 2001a; Hall 1999, 2014). Also, its buffering and remineralizing capacity has the ability to dilute and neutralize acids, forming a diffusion barrier through its acquired pellicle preventing contact between the acids and the tooth structure, providing protection against dental erosion. Several reports (Vukosavljevic, 2014; Hall, 2014) confirm the protective role of the acquired pellicle. The acquired pellicle is a thin film formed once the

enamel surface is exposed to saliva as a result of selective adsorption of salivary proteins, including lipids and glycoproteins. It is formed within seconds forming a protein layer of 10-20nm thick, increasing rapidly to almost 1000 nm. According to Hanning et al., there is no difference in the protective effect after 3 minutes compared to a pellicle formed after 2 hours.

Larsen (2001) demonstrated that fluoride needs calcium and phosphate present in saliva to be able to produce an effective remineralizing process. The lack of all these protective properties becomes very evident on hyposalivation patients (Hara et al., 2014; Schuelter, 2014).

Low salivary flow rate and low buffering capacity not only compromises the ability to remineralize erosive lesions but can also increase the risk of dental erosion. Specifically, decreased unstimulated salivary flow can lead to a low pH level in the mouth below the critical level for enamel (pH 5.5) causing enamel demineralization and compromising the possible reparative effect on eroded lesions. The buffering capacity of saliva plays a critical role in counteracting this temporary drop caused by acidic drinks. It is clear that a reduction in salivary flow can negatively affect the capability to remineralize early erosion lesions. That is why salivary flow stimulation is an important therapy that can show an increase in bicarbonate ions and in salivary mineral content, onto the enamel (Rios et al., 2006; Buzalaf, 2012).

2.3 Patients with Reduced Salivary Flow

The prevalence of patients with salivary gland hypofunction or hyposalivation has increased steadily over the last decades (Billings, 1993; Kyseli, 2016). Meta analysis studies show prevalence from 6% to 46% (Niklander, 2017). This wide range is due to variations in methodology and definitions. The terms hyposalivation and xerostomia are used interchangeably, although they are two different conditions; hyposalivation being the objective measured decrease in saliva flow rate; and xerostomia, the subjective feeling of a dry mouth. The most common cause for salivary gland hypofunction is medication intake. There are more than 400 medications that have dry mouth as a side effect (antihypertensives, antidepressants, diuretics, antiallergics, etc.). It is important to mention that the incidence and severity of hyposalivation is proportional to the number of medications that the patient is taking (Turner, 2016).

It has been reported that other common causes of hyposalivation are Sjögrens disease and head and neck cancer radiation, among others. Despite good oral hygiene, patients with hyposalivation do not have the protective and reparative effect of saliva on teeth, increasing risk for multiple oral diseases, most commonly dental caries and dental erosion (Hannig, 2001; Buzalaf, 2012).

Sjögrens disease formerly known as Sjögrens Syndrome is a chronic autoimmune disorder characterized by lymphocytic infiltration in the moisture producing glands leading to dry mouth, dry eyes, fatigue, joint pain among other symptoms. Sjögrens disease is one of the most prevalent autoimmune diseases

affecting more than 3 million of Americans. The reported prevalence varies from 0.05% to 4.8% and the onset is between 40 and 50 years of age with a female-to-male ratio of 9 to 1 (Turner, 2016). The chronic salivary dysfunction in Sjögrens disease can cause several oral health problems such as dental caries, dental erosion, candidiasis, mucositis, dysphagia, enlarged parotid gland, and difficulty to speak, among others. Sjögrens disease can be present as a primary disease without any other accompanying symptoms (primary Sjögren's disease), and as a secondary disease called secondary Sjögrens disease with other autoimmune diseases such as systemic lupus erythematous, rheumatoid arthritis and systemic sclerosis.

These patients not only suffer from reduced salivary flow but also show qualitative alterations in the saliva such as lower bicarbonate and phosphates and lower degree of saturation with respect to hydroxyapatite (Hall, 1999; da Silva Marques, 2015). These patients experience longer pH drops in their saliva if exposed to acidic challenges which leads to a higher risk of tooth demineralization. A pH drop of less than 5.5 will lead to undersaturation of their salivary calcium and phosphate with respect to hydroxyapatite and results in erosive damage to the teeth.

Different remineralization strategies have been shown to counteract these deficiencies. The most widely accepted strategies for home use are fluoridated toothpastes, which shows overwhelming evidence of its effectiveness, followed by calcium and phosphate compounds as adjunct therapies (casein phosphopeptide-amorphous calcium phosphate CPP-ACP, among others). Most

of the remineralization *in situ* and *in vivo* published studies have been conducted in a standardized healthy population with optimal salivary flow (West, 1998; Zero, 2016). The effectiveness of remineralizing products has not been studied among individuals with chronic reduced salivary flow. Therefore, there is limited evidence to support recommendations for remineralization for patients with chronic reduced salivary flow. In 2016, the Sjögren's Syndrome Foundation along with the American Dental Association (ADA) and its Center for Evidence Based Medicine published the first set of guidelines for oral management in Sjögrens Disease patients (Zero et al; 2016). A panel of experts conducted a systematic review with pre-established parameters to provide guidelines for the management of dental caries in Sjögrens disease patients. The strength of the recommendation was rated based on the quality of the evidence, balance of benefits and harms and cost. Out of the vast available evidence regarding fluoride, only eleven studies were suitable for analysis according to the predetermined criteria, in which only one study looked at Sjögrens patients and the additional studies focused on head and neck cancer patients. In this systematic review as in many others, fluoride received the strongest recommendations as a "first line of treatment". However, recommendations regarding the type of fluoride, its delivery system and its concentration were not possible due to the limited evidence. Regarding calcium-based products only two studies were suitable for analysis making the clinical guidelines weak to moderate due to insufficient evidence (Zero, 2016). The panel concluded that in absence of the evidence and based on experts opinion calcium-based products

can be considered an adjunct to the remineralization therapy. Knowing that our study population is at high risk for dental erosion, it is important to understand if the daily remineralization therapy with topical fluoride and calcium-based agents is sufficient to prevent dental erosion in these individuals with hyposalivation.

Even with the limitation of the available evidence for reduced salivary low patients, fluoride is considered the standard of care for the prevention of caries and dental erosion. For this reason, clinical trials should be designed where all patients receive fluoride as a daily regimen in addition to the test product. According to Zero (2016), there are no studies that compare different fluoride products to prevent dental caries and dental erosion in Sjögrens patients. Therefore, clinical trials are needed to evaluate the effectiveness of fluoride and adjunct remineralizing products in Sjögrens disease patients.

2.4 **Remineralization Strategies**

Remineralization consists on returning the minerals back into a demineralized hard tissue making the tissue more resistant to future demineralization (Cochrane, 2011; Zero, 2012). There are different mechanisms by which a tooth substrate can be remineralized including fluoride and amorphous calcium phosphate (ACP).

Remineralization via Fluoride: Daily home intervention with therapeutic agents such as fluoride has been effective in the prevention of dental erosion (Ganns 2004; Zero, 1996). The concentration of fluoride dentifrices can vary from low 600 PPM to high 5000 parts per million (PPM), being the most common ones

between 1000-1450 PPM. The high concentrations of fluoride are indicated for patients with high risk of caries and dental erosion. Prevident 5000 is a high concentration toothpaste that is commonly prescribed for patients with high risk for caries and dental erosion). Many studies on dental erosion use a variety of fluoride products commonly marketed in the US that have been used over years in caries and erosion prevention such as sodium fluoride (NaF), stannous fluoride (SnF2) and monofluorophosphate (MFP) among others. Ganss et al. (2008) in an *in situ* study reported that fluoride not only reduces demineralization but also enhances remineralization by rehardening a partially demineralized surface. High-concentrated fluoride agents, such as oral rinses, gels or varnishes, have been reported to decrease the development of enamel and dentin erosion *in vitro* and *in situ* (Ren et al., 2009). However, other studies have shown no difference between a 5000 PPM fluoride and a 1000 PPM fluoride dentifrice on eroded enamel (Rios, 2008), suggesting that the efficacy of a fluoridated dentifrice is not necessarily increased by the concentration of fluoride after 1000 PPM. In our study, we intend to compare the enamel resistance to acid attack through 1450 PPM fluoride (positive control) and a high concentration dentifrice with 5000 PPM fluoride on patients with reduced salivary flow, hoping to assist us in proposing suitable prevention strategies for patients with reduced salivary flow. Remineralization via high-concentration fluoride seems to be effective on the prevention of dental erosion.

Remineralization via amorphous calcium phosphate: In 1999, the Federal Drug Administration (FDA) approved MI Paste, a topical paste containing casein

phosphopeptide amorphous calcium phosphate (CPP-ACP) complex, a milk derivative compound for use primarily as a prophylaxis polishing paste and secondarily for the treatment of tooth sensitivity. However, this product has been used extensively as an off-label application for the remineralization of dentin and enamel. The effect of CPP-ACP paste on demineralized dentin and enamel has been demonstrated to promote remineralization of eroded enamel *in vitro* and *in vivo* (Reynolds, 1997, Haghgou, 2017; Thakkar, 2017). Casein phosphopeptide CPP-ACP when in contact with the tooth structure interacts with hydrogen ions and forms calcium hydrogen phosphate, which releases calcium and phosphate ions to the oral environment providing a reservoir of bioavailable calcium and phosphate ions assisting in the remineralization process (Cochrane et al., 2010). By maintaining a state of supersaturation with respect to the hydroxyapatite, these ions depress demineralization and promote remineralization (Srinivasan, 2009).

Casein phosphopeptide CPP-ACP can be delivered in many different ways to the oral environment; among these are chewing gums, lozenges, varnishes and mouthwashes. All of them show to contribute on the remineralization of enamel (Thakkar, 2017). The effectiveness of CPP-ACP as a prevention agent to enhance remineralization has been proven *in situ* models. However, little is known about the effectiveness of these therapeutic pastes against early erosive lesions in patients with chronic reduced salivary flow.

Understanding the efficacy of these two products on patients with chronic reduced salivary flow may help us to provide future guidelines for individuals with this common condition who are at higher risk of developing dental erosion.

2.5 ***In Situ*** **Models and Testing Methods**

The dynamic process of remineralization and demineralization can be studied through *in vitro, in situ* studies and randomized clinical trials. *In situ* models consist on the use of oral appliances that create defined conditions in the oral environment that simulate the process of dental caries or dental erosion. These models vary from removable appliances to single-section fixed appliances (Zero, 1995). *In situ* models have been validated as surrogate models of large randomized control trials showing dose response with different remineralizing agents. (Shellis et al., 2011). There are many advantages to using an in situ model as they are performed in the human mouth. This model requires a relatively small number of subjects and mimics the natural conditions of the human mouth that simulate the process of erosion or remineralization, in the presence or absence of saliva, yet providing control of experimental variables without causing any irreversible damage to the human dentition (Rehder, 2006; Zero, 1998). Different from clinical trials, these studies require only a few months to provide valid results and are statistically efficient as the subject serves as their own control (crossover design). Studies using *in situ* model have shown significant dose response effect from fluoride (Rehder, 2006; Zero, 1998).

The conditions used in *in situ* models can vary with each research trial. Among the experimental parameters to consider in the design of in situ models are the characteristics of the subjects, type of hard tissue substrate, methods of assessing mineral status, and clinical protocol. Regarding erosive challenges, some are performed *in vivo*, facing possible ethical dilemmas with subjects exposing their dentition to acidic drinks, or *in vitro* challenges where the erosive challenge is performed on a tooth substrate outside of the mouth in a standardized way with respect to its acidity, pH, volume, time of exposure, and amount of tissue loss.

Perhaps the most challenging variations in *in situ* models are the characteristic of subjects and the tooth substrate used. The selection of subjects depends on the objectives of the study (i.e. salivary flow rate) and should be representative of the population of interest for which the treatment is intended. Furthermore, subjects' behavior such as oral hygiene and dietary habits can affect the remineralization ability of a product in combination with the biological factors, such as the presence or absence of saliva and the protection from its acquired pellicle.

Regarding the hard tissue substrate, most in situ models use either human or bovine enamel. Human enamel is the most ideal substrate due to its clinical relevance. However, human enamel varies more than bovine enamel in composition due to genetics, exposure to fluoride, diet and environmental conditions (Zero, 1998). Also human specimens are difficult to obtain for research and are small and curved making it difficult to prepare for test

conditions. For these reasons bovine enamel is frequently used as a substitute of human enamel. There are some concerns with regards to its composition and structure. Although very comparable, bovine enamel is structurally different and slightly more porous affecting their speed of erosion (Zero, 2010). In our study, we elected to use bovine enamel blocks as a highly standardized substrate with less variations resulting in less variable response in the situ model. Also due to their large size, bovine teeth allow us to obtain multiple enamel blocks from the same specimen diminishing the variability of the substrate. Reducing variability in remineralization of in situ models is very important to obtain meaningful results. Reducing experimental variability can be accomplished through very meticulous standardization in each step, such as the sample preparation protocol, acidity and pH of the solutions, exposure time extraorally and intraorally, and microhardness test load, leaving variations only related to the subjects inherent characteristics and compliance.

This study used a fixed appliance with continuous presence similar to an orthodontic bracket, which housed a standardized eroded bovine enamel slab cemented in the mouth, eliminating the factor of subject compliance with regards to wearing the appliance. This fixed model offers the advantage over the removable model of enabling the specimen to be subjected to all the daily processes and function occurring within the oral cavity. Also, the test location is important as the remineralization response can be influenced by the position of the substrate in the mouth due to the proximity to the major salivary glands, the rate of salivary flow and anatomical factors. Three different sites have been

suggested: the palatal surface of maxillary anterior teeth, the buccal or lingual surface of mandibular molars, being the latter, the most responsive to remineralization due to its constant exposure to saliva in the area (West, 2011). In this experiment we chose the mandibular molars as a standard test site as oppose to the preferred lingual surfaces, due to patients' comfort and protection of the enamel slab by the buccal mucosa, yet providing proximity to sublingual and submandibular glands as well as saliva availability.

There are several ways of measuring the outer changes and mineral content on enamel such as transverse microradiography (TMR), surface microhardness test, profilometry and polarized microscopy. Several studies (Lippert, 2017; Mathews et al., 2012) demonstrated that surface microhardness (SMH) offers excellent agreement with TMR. Surface microhardness intend to measure mineral loss or gain, while TMR measures mineral content complementing each other. Zero (1995) reported that for early erosive changes on the enamel, like the ones produced in this experiment, surface microhardness test is highly correlated with remineralization as it has been validated by microradiography. Surface microhardness tests using either Vickers or a Knoop indenter are widely used on in situ models as it offers excellent sensitivity to detect early changes in the enamel surface (Zero et al., 1998). Due to the variations observed in outcome measures with in situ models, it may be necessary a combination of assessment methods to increase predictability of the clinical outcome.

3. MATERIALS AND METHODS

3.1 IRB Approval

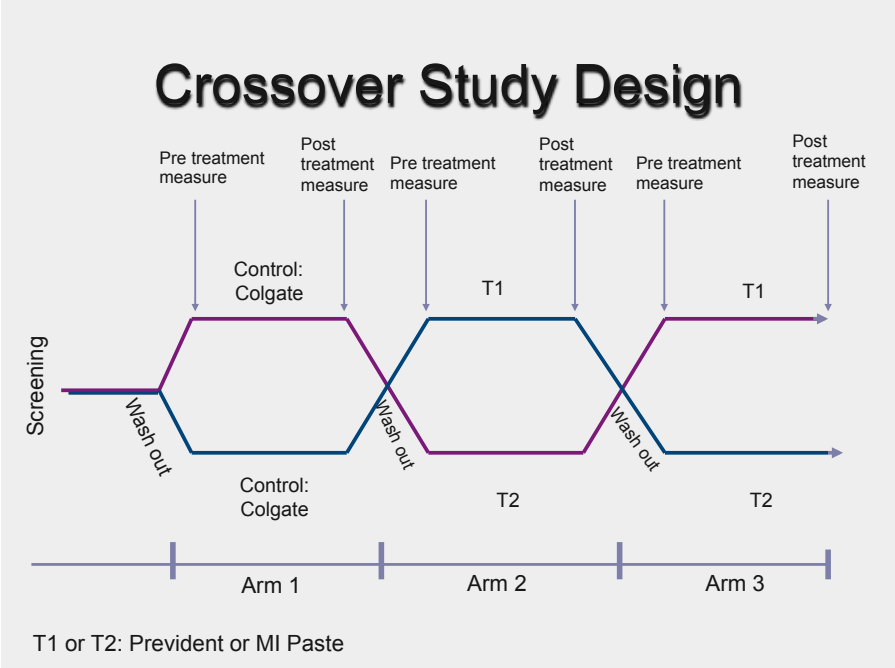
The study was approved by the Institutional Review Board (IRB), at the University of Illinois at Chicago (Approval # 2010-0597) on November 8th, 2010, Appendix A. Recruitment material is found in Appendices B and C. All data were entered in a timely manner once collected in Excel sheets (Appendix D). The database is password protected and housed in a secure location. At the completion of the data collection, data was entered into a SPSS file for statistical analysis.

3.2 Study Design

This *in situ* study was conducted in a double blind cross-over design with all subjects completing three arms (Figure 1). The study involved the use of a fixed in situ remineralization model to evaluate the ability of 2 different dentifrices and 1 positive control dentifrice use for at-home therapy to enhance remineralization of enamel that was previously subjected to an in vitro erosive challenge. Twenty-one subjects between 18 and 64 years old participated in 7 visits involving 2 experimental dentifrice treatments and one control phase: T1: PreviDent 5000 Plus (Colgate-Palmolive Co., New York City, NY, USA) (1.1 % NaF), and T2: MI paste (Recaldent® with CPP-ACP complex). The initial control phase involved the use of a dentifrice for daily use; Colgate Cavity Protection (0.76% Sodium monofluorophosphate, MFP). The order of dentifrice treatment was randomly

assigned to each of the subjects always starting with the use of Colgate Cavity Protection toothpaste alone as the first phase. Each treatment lasted for a total of 2 weeks starting with 7 days of washout period, followed by 7 days of a remineralization period. For the washout period the subjects were instructed to continue with their routine oral hygiene using only Colgate Cavity Protection toothpaste. (i.e. brushing a minimum of two times daily for two minutes on each time, preferably morning and before bedtime).

Figure 1. Crossover Study Design



3.3 **Study Population**

The population of interest was subjects with chronic salivary gland hypofunction caused by Sjögrens disease with the exception of a head and neck cancer therapy patient (radiotherapy), as well as healthy subjects with normal salivary flow. Salivary gland hypofunction was determined by the following criteria:

(A) Subjective symptoms: a patient questionnaire about symptoms of oral dryness and compromised oral functions related to saliva secretion was part of the medical history collected. Positive responses to the following four questions are associated with salivary gland hypofunction: (Navazesh et al., 2008):

- Does the amount of saliva in your mouth seem too little?
- Does your mouth feel dry when eating a meal?
- Do you have difficulties swallowing any food?
- Do you sip liquids to aid in swallowing dry food?

(B) Objective measurements included: unstimulated whole saliva flow ≤0.2 ml/min and, stimulated salivary flow rate ≤0.8 ml/min. Values of > 0.3 ml/min for the unstimulated salivary flow rate and > 0.9 ml/min for the chewing stimulated one are considered normal for healthy individuals.

The inclusion criteria consisted of:

For normal salivary flow subjects: subjects willing to give a full medical and drug history with at least 20 teeth exposed to the oral environment, with no current clinically observed cavitated carious lesions**;** have unrestored enamel on

the buccal surface of at least one lower first or second permanent molar which carried the in situ appliance.

For reduced salivary flow subjects: Subjects who suffer a reduction of unstimulated salivary flow rate below 0.2 ml/min, and a stimulated salivary flow rate below 0.8ml/min due to medications, a systemic disease or radiation therapy.

Subjects were excluded if they exhibited current active caries or active periodontal disease, were pregnant or breastfeeding and have suspected allergies to the test products.

An analysis based on the descriptive statistics of a published article about hyposalivation (Niklander et al., 2017) indicated that a sample size of approximately 10 subjects per group would be sufficient to achieve at least 80% of a power with 5 % error type I to detect a mean salivary flow rate difference between two study groups.

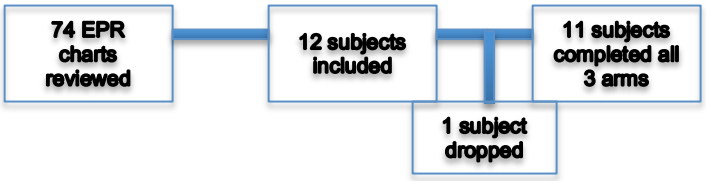
Based on the study criteria, twenty-two subjects were available to the research and provided their signed consent to participate. One female patient never started treatment due to health reasons. Twenty-one adults, 2 males and 19 females, ages between 18 and 64 years old, completed all three arms of the study; 10 with normal salivary flow and 11 with reduced salivary flow.

3.4 **Recruitment**

Recruitment methods included the use of flyers, emails to UIC College of Dentistry staff and a physician's letter. (Appendix B).

For reduced salivary flow subjects recruitment, the Sjögren's Syndrome Foundation (SSF) directly distributed a UIC IRB approved flyer containing information about the study via mail to their members in the city of Chicago and near suburbs. Also a search through UIC electronic patient dental records (AxiUm) was performed to identify additional patients from the College of Dentistry (Shown in Figure 2).

Figure 2. Reduced salivary flow subject recruitment



Normal salivary flow subject recruitment

3.5 Data Collection

A. In Vitro phase (Enamel preparation): A modified protocol for enamel preparation was used in this study:

Specimens obtained from bovine permanent central incisors teeth of commercially sacrificed cows were included as the hard tissue substrate. Teeth were selected based on the quality of the enamel surface. Specimens with,

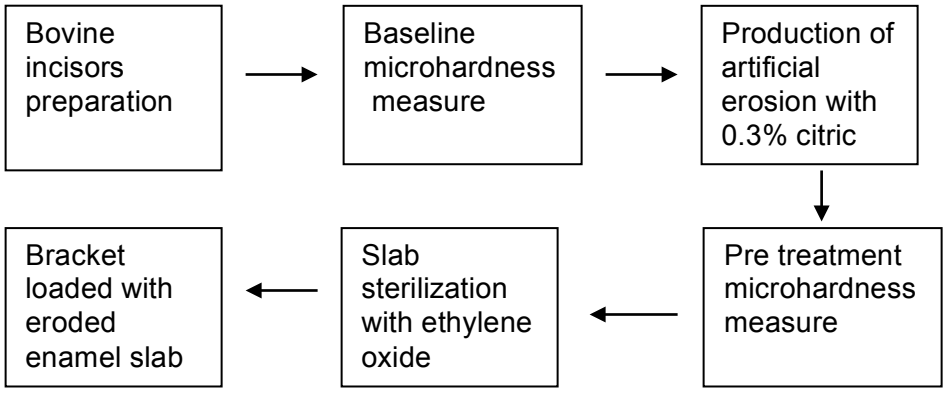
cracks, signs of demineralization, white spots and other defects were rejected. The tooth slabs were cut using a sectioning machine (Isomet 1000, Buerler, Lake Bluff, IL, USA). Three or four enamel blocks were produced from each tooth measuring approximately 2 mm length x 2 mm width x 1.5 mm thickness. The teeth were stored in 0.1 % thymol solution during the sample preparation process.

In order to ensure reliable surface microhardness testing (SMH) measurements, the enamel surface of each specimen was polished flat as described by Zero et al. (1990). The specimens were ground and polished to create planoparallel surfaces to facilitate surface microhardness testing using an Isomet 1000 sectioning machine. The specimens were serially polished using 2400, 4000 aluminium oxide grit paper followed by 1 μm diamond polishing suspension on a polishing cloth. The specimens were assessed under the stereomicroscope at 10x magnification.

The baseline surface microhardness was tested on each block surface using a Knoop diamond indenter (Confident2 with a Leco Microhardness Tester LM 700AT), with a load of 50 grams (gr) applied for 15 seconds. Three indentations were made at the middle, upper and lower ends of the enamel surface (preserving a reasonable sound area of 100 microns (μms) between the indentations. The baseline SMH (SMHb) was determined by measuring the length of the indentations (μm) using an image analysis system interfaced with the LECO LM 700 microhardness tester. The image was transmitted through the use of a video camera to the monitor of the computer. Enamel slabs inclusion

was based on mean indentation length at baseline of 52 μm ± 6μm. A total of 100 bovine enamel slabs were randomly divided into the 3 experimental groups. Then, an early erosive enamel lesion was created on each block by 7 minutes exposure to 0.3 % citric acid (pH 3.75) and SMH was measured again obtaining the pretest SMH (SMH1). Before insertion into the subjects' bracket appliance, all enamel specimens were sterilized by ethylene oxide gas, as it does not affect the structural integrity of the enamel block (West, 2011). The following graphic (Figure 3.) summarizes the *in vitro* phase:

Figure 3. In vitro phase



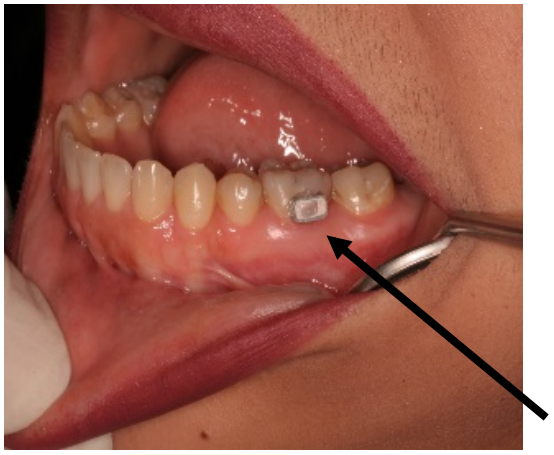
B. In vivo Intraoral phase (interventions):

The study comprised of 3 arms during which subjects were exposed to the following treatments in a double blind randomized crossover design:

- A high concentration prescription strength fluoride dentifrice Prevident 5000 (1.1% NaF, Colgate Palmolive) in combination with a 1450 PPM dentifrice,
- A calcium-based product MI Paste (Recaldent-CPP-ACP, GC America®) in combination with a 1450 PPM dentifrice,
- An over the counter (OTC) positive control dentifrice containing 1450MFP (Colgate Cavity control Colgate Palmolive) alone.

A fixed *in situ* appliance (orthodontic bracket) was used to carry the bovine enamel block on the buccal surface of a mandibular molar of the subject. The appliance is based on the design of brackets used in orthodontics, and consists of a custom-made bracket with an orthodontic molar pad and retentive mesh backing, which has a rectangular stainless steel band welded to it to form a box within which the test block is retained using Intermediate Restorative Material (IRM® Dentsply Caulk) cement (fluoride–free). One bracket was cemented per subject. All appliances loaded with enamel slabs were sterilized with ethylene oxide prior to intra-oral application.

Figure 4. ***In situ*** **(intraoral) appliance (Picture courtesy of Dr. Ben Amaechi)**



All subjects completed a total of 7 visits of 1-2 hours each to complete all three study arms (Figure 1). During each arm, subjects went home with a cemented bracket appliance containing an *in vitro* eroded enamel bovine slab and written instructions for remineralization dentifrice treatment for a period of one week. All subjects were asked to maintain their normal dietary habits; however, the use of any other oral hygiene product (like mouthwash or whitening products) was prohibited. These measures were to ensure uniformity in the use of oral hygiene product, which may influence the de-/remineralization cycle during the study periods. The importance of good oral hygiene while wearing the appliance was emphasized. A special toothbrush was provided for use with orthodontic brackets to facilitate brushing properly around the appliance, not on the appliance. A log was given to all subjects to enter their hygiene regimen including the time of the day.

After 1 week of treatment, the subject returned to UIC clinics and the enamel block was removed from their respective appliance. SMH was measured by making three new indentations on the eroded slab. The values from the three indentations were average for each block obtaining the post test SMH (SMH2). This protocol was repeated 3 times for all three arms. The order of dentifrice treatment was randomly assigned to each of the subjects. Subjects were blind to the dentifrice received. Each treatment lasted for a total of 2 weeks starting with 7 days of washout period, followed by 7 days of a remineralization period. For the washout period the subjects were instructed to continue with their routine oral hygiene (i.e. brushing a minimum of two times daily for two minutes on each time, preferably morning and before bedtime).

The percentage SMH recovery was determined after the in vitro erosive challenge pre treatment (SMH1) and, after *in situ* remineralization, post treatment (SMH2).

3.6 **Measurements**

Percentage Surface Microhardness Recovery (%SMH): Microhardness tests are commonly used to test physical properties of materials. This method requires a small surface for testing in which the surface is impressed with a diamond indenter (Knoop or Vickers) at a specific load for a certain period of time and the length of indentation left is measured with an optical microscope.

Once subjects completed a week of treatment, the enamel blocks were removed from their respective appliances, and the surface microhardness post-

test was performed by three indentations on the free (un-indented) surface of the enamel block, and the average value was calculated for each block.

At this point the pre-treatment (SMH_1) and post-treatment (SMH_2) surface microhardness values (SMH) of the lesions were available. Higher values indicate higher microhardness recovery. The percentage SMH recovery was determined by the following formula:

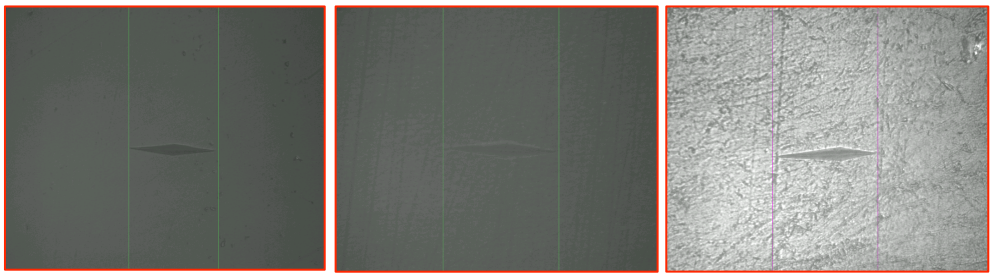
Percentage SMH=(SMH1-SMH2/SMH1-SMHB) x 100

SMHB= indentation length (μm) of sound enamel

SMH1= indentation length (μm) after in vitro erosive challenge

SMH2= indentation length (μm) after in situ remineralization

Figure 5. Representative images of an enamel slab after Knoop baseline (SMHB) indentation measurement, post-erosive challenge (SMH1) and post-remineralization (SMH2).



SMHB **SMH1** **SMH2**

Sialometry: To study the possible saliva and remineralization response, stimulated and unstimulated saliva were collected and stored under standardized conditions using methods described by Screebny and Valdini (1987). Patients were instructed to refrain from eating, drinking, smoking or any factors being used to influence saliva flow for at least two hours prior to collection of saliva. Stimulated saliva was collected during the second arm; visit 5 (removal of the enamel slab) for 10 minutes with an expected volume of approximately 10 ml for healthy subjects and < 5 ml for impaired salivary flow subjects. Subjects were instructed to chew on unflavored gum base for 2 minutes and to swallow any pooled saliva. They continued to chew unflavored gum base for an additional 10 minutes, during this time pooled saliva was collected into a collection tube. Unstimulated saliva was collected during the 3rd arm; visit 7 (removal of the last enamel slab) by passive drooling into a container for 10 minutes. The collected saliva was measured to obtain salivary flow rate (ml/min), also the unstimulated saliva pH was determined for each subject. Subject's whole saliva were stored at -80°C for future analysis of inorganic compounds and proteins.

3.7 **Statistical Analysis**

Exploratory data analysis indicated that the continuous variables have an approximately normal distribution. A two-way ANOVA was conducted to assess the main effect of three remineralization agents and the saliva secretion rate (normal versus reduced saliva flow) on percentage surface microhardness recovery.

Student t-test for paired samples was conducted to compare the difference in remineralization between SMH_1 and SMH_2 data. Levene test was computed to test the assumption of homogeneity of variances among groups.

Statistical significance level was set at 0.05 for all tests. The software used for the data analysis was IBM SPSS (version 22.0, IBM Corp., Armonk NY).

4. RESULTS

4.1. Data Analysis

4.1.1. Subjects Demographic

A total of 21 subjects participated in the study (Table I). The study visits occurred from November 2010 to November 2011. Of the 21 subjects, 10 were included in the normal salivary flow group, and 11 met the inclusion criteria for the reduced salivary flow group. Out of these 11 subjects with reduced salivary flow, 10 subjects have Sjögrens disease and 1 had head and neck cancer radiotherapy. All 21 subjects completed all 3 remineralization treatments with a 1-week wash out period. One subject lost one enamel slab missing one of the three arms (MI Paste). No product related adverse events were reported and there were no deviations from the study design. The demographic characteristics of the subjects are summarized in Table I.

4.1. 2. Percentage Surface Microhardness Recovery

Exploratory data analysis indicated that the continuous variables have an approximately normal distribution. Comparison of percentage SMH recovery for both normal salivary flow and reduced salivary flow group was calculated using two-way analysis of variance (ANOVA). Table II illustrates the mean surface microhardness recovery by treatment for normal and reduced salivary flow subjects.

TABLE I.

DEMOGRAPHICS DISTRIBUTION OF SAMPLE

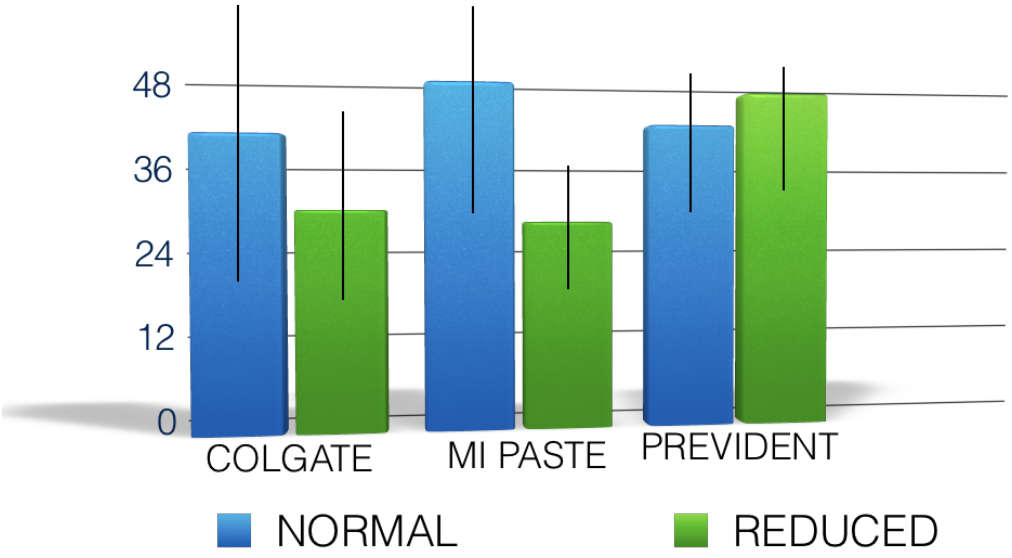
Categories of subjects	Dry salivary flow group (n=11)	Normal Salivary flow group (n=10)
Gender		
F	10	9
M	1	1
Age in years (Mean, SD)	(51, 9)	(48.9, 12.5)
Ethnicities		
Asian	2	0
African American	2	1
Caucasian	4	5
Hispanic	3	4
Unstimulated Saliva flow ml/min (Mean, SD)	0.17, 0.12	0.63, 0.39

TABLE II.
MEAN SURFACE MICROHARDNESS (SMH) RECOVERY BY TREATMENT (%)

Salivary Flow	Treatment	N	Mean SMH recovery	Std. Deviation
Normal	Colgate	10	40.45	37.77
	MI Paste	10	47.66	27.76
	Prevident	10	42.01	23.73
	Total	**30**	**43.37**	**29.44**
Reduced	Colgate	11	30.19	30.89
	MI Paste	10	28.51	20.91
	Prevident	11	46.57	24.87
	Total	**32**	**35.30**	**26.52**
Total	Colgate	21	35.07	33.86
	MI Paste	20	38.09	25.86
	Prevident	21	44.40	23.84
	Total	**62**	**39.20**	**28.04**

The two-way ANOVA showed not statistically significant results, p-value>0.05. These results indicated the absence of main effects and interaction for remineralization and salivary flow type, for percentage surface microhardness recovery variation, F (2,56) = 0.927. p-values=0.402. The assumption of homogeneity of variances was not violated P=0.767. Therefore, the null hypothesis 1 was accepted. This means that no difference was observed in the percentage surface microhardness recovery, not been able to discern the effect of reduced salivary flow on the remineralization treatment on eroded enamel. The results for percentage SMH recovery are illustrated in Figure 6.

Figure 6. Graphical representation of the percentage SMH recovery for normal and reduced salivary flow groups



In this investigation, all three treatments resulted in measurable increase in enamel microhardness recovery following a 1-week, at-home use of these dentifrices. The microhardness recovery was not influenced by the salivary flow rate grouping of subjects. Regarding salivary flow, the normal group, showed a 40% surface microhardness recovery and the dry group showed a 35% SMH recovery.

While no statistically significant difference was observed between the different dentifrices, Figure. 6 suggests that Prevident 5000 + Colgate Cavity Protection showed a higher mean recovery showing a mean SMH recovery of 46.57% compared to 30.19% recovery for Colgate alone and 28.51% for MI Paste+ Colgate Cavity Protection.

Paired sample student t-tests at each pair remineralizing agents: Colgate, MI Paste and Prevident group, showed statistical significant mean differences between the pre test and post test for both salivary flow conditions groups (normal and dry), which indicated a significant gain in mineralization following exposure to each remineralizing agent, (p-values<0.05). Therefore, hypothesis 2 was rejected.

At the end of 1 week of remineralization treatment *in situ,* all three groups experienced some degree of remineralization. The reduction of the mean indentation length (μm) after treatment is shown in Table III and indicates an increase in surface hardness due to the effect of the remineralizing agents.

TABLE III.

STUDENT PAIRED T-TEST RESULTS FOR SURFACE MICROHARDNESS

Variables	N	MEAN, SD μm	MEAN DIFF μm	STD. error mean	95% C.I. Lower, Upper	t	df	p-values
NORMAL SALIVARY FLOW								
Colgate PRE	10	67.98, 6.58	6.07	2.01	(1.51, 10.63)	3.01	9	0.015*
POST		61.90, 3.37						
MI Paste PRE	10	64.57, 7.51	6.59	1.68	(2.78,10.40)	3.91	9	0.004*
POST		57.97, 3.45						
Prevident PRE	10	64.28, 3.96	5.46	1.05	3.07, 7.85)	5.17	9	0.001*
POST		58.82, 2.80						
DRY SALIVARY FLOW								
Colgate PRE	11	63.98,4.65	4.08	1.36	(1.05, 7.12)	3.0	10	0.013*
POST		59.89, 5.10						
MI Paste PRE	10	64.66,6.65	3.91	0.98	(1.69, 6.14)	3.97	9	0.003*
POST		60.74,5.36						
Prevident PRE	11	65.25, 3.23	5.87	0.93	(3.78, 7.96)	6.26	10	0.000*
POST		59.37, 4.26						

*Statistically significant at 0.05

4.1.3. Sialometry

Additionally, independent student t-test showed that the mean of normal salivary flow group in ml/min (mean=0.47 ml/min; SD=0.407) is highly statistically significant from the mean of the dry group (mean 0.17 ml/min; SD=0.127) with statistically significant mean differences of 0.32, p value=0.049.

Since reduced salivary flow has been identified as a risk factor for dental erosion (Amaechi et al., 1999a), it was important to understand the secretion rates of both normal and dry salivary flow subjects. These findings helped us confirm the inclusion criteria for both groups. It also allowed us to learn the effect of saliva (or lack of) on dental erosion.

5. DISCUSSION

5.1. Summary of Methods and Findings

This double blind, crossover, 3 arms study involved the use of a fixed *in situ* remineralization model to evaluate the ability of 3 different remineralization therapies: a high concentration fluoride dentifrice, Prevident 5000 + 1450 PPM fluoride dentifrice, a calcium based topical paste, MI Paste + 1450 PPM fluoride dentifrice, Colgate Cavity Protection MFP fluoride, 1450 PPM alone (positive control). Twenty-one subjects holding an acid eroded enamel slab brushed their teeth with the test dentifrices for one week in each phase with one week of washout period. Based on the available sample, no difference in microhardness recovery was observed between the three treatments regardless of the salivary flow and the age of the subjects. This study was the first investigation comparing the difference in surface microhardness recovery between reduced salivary flow patients, specifically Sjögrens disease subjects and normal salivary flow subjects in an in situ model.

In a similar *in situ* model, Hara et al. (2009) demonstrated the positive influence of two fluoridated dentifrices (1,450 PPM) and a placebo dentifrice (0 PPM) on the remineralization of eroded lesions. Hara's study showed superior remineralization for the fluoridated dentifrices of 36% compared to the placebo of 22% hardness recovery, suggesting that the level of remineralization was affected by the concentration of fluoride. This study had a large sample size of 58 subjects with normal salivary flow and a mean age of 29 years of age. In

contrast, other study with 10 subjects and normal salivary flow showed no difference between a 5000 F and 1000 PPM NaF dentifrices on eroded enamel (Rios, 2008), suggesting the efficacy of a fluoridated dentifrice was not necessarily increased by the concentration of fluoride after 1000P PPM.

As the majority of *in situ* models have included normal salivary flow subjects, it was important to explore the impact of reduced salivary flow rate on enamel remineralization at the level of microhardness. In our experiment, two groups with significantly different measured salivary flow rates benefited from the in vivo tested remineralization protocols. While not statistically significant, two dentifrice protocols showed a trend for enhanced remineralization in participants in the normal salivary flow rate group compared to the reduced salivary flow group.

In our experiment, a fluoridated toothpaste (1,450 PPM) was used as a positive control and as part of their daily oral hygiene in combination with the other two remineralization treatments, because it is the standard of care for the population of interest due to their increased risk for dental caries and dental erosion. For this reason, placebo toothpaste with no fluoride was not ethically permissible. The other two remineralizing agents, Prevident 5000 PPM (44%) and MI PASTE (38%), in combination with the 1450 PPM dentifrice, achieved a similar percentage SMH recovery compared to the positive control alone (35%). The mean percentage SMH recovery values for both salivary groups were in agreement with other in situ models (Hara et al., 2009; Mathews, 2012) failing to demonstrate the influence of saliva in the process of remineralization. Although no mean difference on percentage SMHR was observed among the three

treatments, Prevident 5000 + Colgate Cavity Protection exhibited a trend towards higher microhardness recovery. One possible explanation for the lack of difference of the groups was the small sample size in each group (10 versus 11), the heterogeneity of the subjects participating in the study, and the variations relative to the *in situ* model.

Our population of interest were patients who suffer from chronic salivary flow mainly due to Sjögrens Disease with a mean age of 50 years. Patients with Sjogrens disease suffer from a number of symptoms and medical conditions that require the intake of multiple medications that can worsen their salivary secretion rates. A new finding observed with our results, under the conditions of this model, is that all subjects in the reduced salivary flow rate yielded a remineralization response comparable to other studies testing a healthy population with normal salivary flow (Mathews, 2008).

As expected in clinical trials or in situ models, we can speculate that in this experiment, subject heterogeneity impacted the surface microhardness recovery for all treatments. In order to reduce the variability between subjects in our study, we carefully considered a strict inclusion criteria. Our inclusion criteria required recruitment of reduced salivary flow patients in relative good health with no signs of active caries or periodontal disease with al least 20 teeth present, which is uncommon to observe in this high-risk population, making the recruitment process very challenging. Also subjects were controlled for their oral hygiene products during the experiment as well as their brushing technique around the brackets. However, behavioral aspects related to diet and frequency

of acid intake were not controlled resulting in possible variability of the remineralization response for all treatments. For example, some of our patients could have used citric fruits to stimulate their saliva flow. Following the slab retrieval and likely due to patient's daily oral hygiene habits, some enamel slabs showed the presence of scratches and defects that made the Knoop indentation measurement difficult due to undefined endpoints of the surface indentation during hardness testing. Although patients were asked to follow the same oral hygiene regimen, it is possible that the mechanical action of brushing could have abraded the eroded enamel slab.

In addition, Piangprach et al. (2009) indicated that age is an important factor on dental erosion as salivary flow of older subjects may have an altered salivary flow rate and composition. In average, our participants were older than previous studies, possibly affecting the effect of the remineralization agents tested (Wegehaupt, 2012; Hara et al., 2009; Mathews, 2012).

An important parameter to discuss related to the *in situ* model is the intraoral location of the enamel slab. Amaechi and Higham (2001a) showed that the extent of the remineralization differs by the location of the intraoral device in the mouth. He demonstrated that eroded enamel slabs located on the lingual surface of the lower incisors presented higher degree of remineralization because those areas are constantly bathed by a pool of saliva from the submandibular and sublingual glands. This can infer that the variability in remineralization response is due to the constant presence of saliva providing calcium, phosphate and fluoride ions reducing the severity of erosion in the

dental arches. In our model and because of patient's comfort, the fixed appliance were placed on the mandibular molars as opposed to the lingual surfaces, still providing proximity to sublingual and submandibular glands.

As mentioned in the literature review, the experimental parameters used in *in situ* models can vary with each research trial. Among the most challenging to control is the type of hard substrate. The substrate in this study was bovine enamel, which has been widely accepted as a surrogate for human enamel. During the *in vitro* phase of this study, all enamel slabs were prepared with a standardized protocol to produce early erosive lesions by 7 minutes of exposure of citric acid at a pH of 3.75. The heterogeneity of the samples was reduced by only including those slabs that showed a mean indentation length of 52-μm ± 6μm, and that were able to respond to the demineralization process. However, we still found large variations in the microhardness measure within the same slabs even when we did every measure within 100 μm of distance from each other.

Other important parameter to discuss is the type of measurement used in *in situ* models. The measurements used in remineralization studies vary, such as 3D scanning microscope, a stylus profilometer and transverse microradiography among others (Lippert, 2017). Along with surface microhardness, all these tests are useful to understand the effectiveness of the remineralizing dentifrices. We chose Knoop surface microhardness over other measures as it has shown good sensitivity for early eroded lesions (Nehme, 2016) as the ones created artificially in this experiment. Additionally, surface microhardness tests have shown to be

sensitive to the remineralizing agents used in our study to detect rehardening of the bovine eroded enamel slabs (Nehme, 2016). The measure used depends on the aim of the study. Many studies use more than one measure to validate their findings. In this experiment Knoop hardness was the only measure.

We found no mean difference on percentage SMHR among the three treatments, however Prevident 5000 in combination with Colgate Cavity Protection exhibited a trend towards higher microhardness recovery. Prevident 5000 is a commonly prescribed product for patients suffering from dry mouth that shows overwhelming evidence on its effectiveness on a healthy population (Ren et al., 2009), but limited evidence on its effectiveness in patients with chronically reduced salivary flow. Ren et al. (2009) reported in an *in vitro* study that remineralization treatment with a high concentration fluoride (Prevident 5000) could increase enamel resistance after an erosive challenge.

As mentioned in the previous section, CPP-ACP is available in many different delivery systems (dentifrices, chewing gums, lozenges, varnishes) that have shown to contribute on the remineralization of enamel in vitro and in vivo. We chose MI Paste as it has the benefit of being in direct contact with the tooth structure, compared to other home delivery systems. Some studies have demonstrated significant hardening of the enamel after erosion with soft drinks by using a paste as a delivery system. In contrast other studies failed to demonstrate its protective effect on erosive lesions (Wegehaupt, 2011).

An *in situ* study by Prestes (2013), analyzed the effect of CPP-ACP in chewing gum on eroded enamel resulting in similar microhardness recovery

compared to our experiment. In Prestes's study, twelve healthy subjects with normal salivary flow wore a palatal appliance for 1 hour resulting in a significant microhardness recovery of 30% (chewing gum with CPP-ACP) compared to 20% (chewing gum without CPP-ACP) and 10% (no chewing gum). The significant recovery observed in both chewing gum treatments could be explained by the increase salivary flow, as saliva has a positive effect on remineralization, and this can overestimate the effect of the chewing gum alone.

As mentioned earlier, other studies have failed to demonstrate the protective effect of MI Paste on erosive lesions (Wegehaupt, 2011). One of the reasons for these findings is that the acquired pellicle in saliva can have a barrier effect that prevents the diffusion of the topical paste to the enamel. For these reasons it is important to account for saliva's interaction with the paste and the oral structures when studying these models.

In our study, the available sample was 11 patients with reduced salivary flow and 10 patients with normal saliva flow matched on age and gender. According to the power of analysis based on Niklander et al. (2017), this sample size was sufficient to achieve at least 80% of a power with 5% error type I to detect a mean salivary flow rate difference between two study groups. However this sample size may have not provided enough power to observe a statistical significant difference in microhardness recovery between salivary flow groups for each treatment group. A modification to this model could include increasing the number of subjects in each group. This study provided pilot data that could guide

future studies. Data from larger clinical studies are needed to develop specific practice guidelines for dental erosion prevention in SD patients.

5.2 Strengths and Limitations of the Study

There are limited data on the influence of remineralizing agents in the erosion process when salivary flow is impaired. To our knowledge this was one of the first studies to evaluate the efficacy of remineralizing agents on a reduced salivary flow population, specifically in Sjögrens patients. This group of patients has not been included in remineralization studies, and is in high need of effective recommendations to prevent dental erosion.

A possible modification of the study would be to add more than one enamel block per patient to assess if the location site of the enamel slab affects the remineralization ability. Additionally, we can consider the addition of other measures complementary to the microhardness test such as microradiography to add information regarding the mineral density of each enamel slab after each treatment.

This study will assist in more accurate recommendations to prevent and treat dental erosion. Additionally, the crossover designs made the study statistically efficient and so required fewer subjects than do non-crossover designs.

Recruitment and retention was a limitation of this study. Although reduced salivary flow patients are highly prevalent, it was challenging to recruit and retain individuals with no active caries and no periodontal disease who present with this oral condition due to Sjögrens disease. This group of patients presented with

many other health problems that could have affected their participation on the study. As the remineralization treatment was heavily dependent on patient compliance, all subjects were asked to complete a daily log and also received phone calls to remind the subjects about the study protocol.

5.3 **Future Research**

To better understand the kinetics of remineralization products in high risk populations, more research is needed, especially large prospective clinical studies involving salivary factors linked to dental erosion. It is important that future studies include patient's behavior, like eating and brushing habits that can affect erosion.

A variety of parameters can be standardized in *in situ* models (tooth substrate and its location in the mouth, erosive challenge, exposure time and pH, hardness tester load and dwell time) making a reproducible model with less outcome variability. There is need for future studies to develop consensus on the parameters of the *in situ* model, and the variables used to measure remineralization.

The individual's heterogeneity will be difficult to minimize, but each experiment should take these variations into consideration. Future studies may lead to more effective prevention and treatment of tooth erosion in patients with hyposalivation.

6. CONCLUSIONS

Within the limitations of this study, it can be concluded that a differential effect of remineralizing agents was not discernible between reduced salivary flow and normal salivary flow subjects on early enamel erosion.

In addition, we were able to observe that a 1,450 PPM fluoride dentifrice alone, and in combination with a 5000 PPM prescription strength fluoride dentifrice and a calcium-based product were able to enhance remineralization in both normal and reduced salivary flow patients with a trend towards better remineralization response for the 5000 PPM fluoride dentifrice.

CITED LITERATURE

Amaechi BT, Higham SM, Edgar WM. Use of transverse microradiography to quantify mineral loss by erosion in bovine enamel. Caries Res. 32(5): 351-356, 1998.

Amaechi BT, Hingham SM, Edgar SM. Factors influencing the development of dental erosion in vitro: enamel type, temperature and exposure time. J. Oral Rehab. 26: 624–630, 1999a.

Amaechi BT, Hingham S.M, Edgar WM, Milosevic M. Thickness of acquired salivary pellicle as a determinant of the site of dental erosion. J. Dental Res. 78: 1823–1830, 1999b.

Amaechi BT, Higham, S. Eroded enamel lesion remineralization by saliva as a possible factor in the site-specificity of human dental erosion. Arch of Oral biology. Vol 46, Issue 8: 697-703, 2001a.

Amaechi BT, Higham, S. In vitro remineralization of eroded lesions by saliva. J Dent. Vol 29: 371-376, 2001b.

Bardow A, Nyvad B, Nauntofte B. Relationships between medication intake, complaints of dry mouth, salivary flow rate and composition, and the rate of tooth demineralization in situ. Arch of Oral Biology 46: 413–423, 2001.

Barlow A, Sufi F, Mason S. Evaluation of different fluoridated dentifrice formulations using an in situ remineralization model. J Clin Dent. 20:192-198, 2009.

Bartlett D, Smith B, Wilson R. Comparison of the effect of fluoride and non-fluoride toothpaste on tooth wear in vitro and the influence of enamel fluoride concentration and hardness of enamel. BR Dent J. 176: 346-348, 1994.

Billings R. An epidemiologic perspective of saliva flow rate as indicators of susceptibility to oral diseases. Oral Bio Med. 4(3/4): 351-356,1993.

Buchalla W, Attin T, Schulte-Monting J, Hellwig E. Fluoride uptake, retention, and remineralization efficacy of a highly concentrated fluoride solution on enamel lesions in situ. J Dent Res. 81(5): 329-333, 2002.

Buzalaf MA, Hannas AR, Kato MT. Saliva and dental erosion. J Appl Oral Sci. Sep-Oct. 20(5): 493-502, 2012.

Chanya Chuenarrom, Pojjanut Benjakul, Paitoon Daosodsa. Effect of indentation load and time on knoop and vickers microhardness tests for enamel and dentin. Materials Research. Vol. 12, No. 4: 473-476, 2009.

Cochrane N, Saranathan S, Cai F, Cross K, Reynolds E. Enamel subsurface lesion remineralisation with casein phosphopeptide-stabilized solutions of calcium, phosphate solutions. Caries Res. 42: 88-97, 2008.

Cochrane NJ, Cai F, Huq N.L, Burrow M.F., Reynolds E.C. New approaches to enhanced remineralization of tooth enamel. J Dent Res; 89(11): 1187–1197, 2010.

Da Silva Marqus DN, da Mata AD, Patto JM, Barcelos FA, de Almeida Rato Amaral JP, de Olivaira MC, Ferrerira CG. Effects of gustatory stimulants of salivary secretion on salivary pH and flow in patients with Sjögren's syndrome: a randomized controlled trial. J oral Pathol Med. Nov;40(10):785-92, 2011.

Davis W, Winter P. Dietary erosion of adult dentine and enamel. Protection with a fluoride toothpaste. Br Dent J. 143: 116-119, 1977.

Fushida C, Cury J. In situ evaluation of enamel-dentin erosion by beverage and recovery by saliva. Rev Odontol Univ Sao Paulo 13: 127-134, 1999.

Ganss C, Klimek J, Brune V, Schurmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res. 38:561-566, 2004.

Gelhard TB, ten Cate JM, Arends J. Rehardening of artificial enamel lesions in vivo. Caries Res, 13. 80-83, 1979.

Haghgou EH, Haghgoo R, Roholahi MR, Ghorbani Z. effect of Casein phopohopeptide-amorphous calcium phosphate and three calcium phosphate on enamel microhardness. J Contemp Dent Pract. Jul 1;18(7):583-586, 2017.

Hall A, Buchann C, Millet S, Creanor S, Strang R, Foye R. The effect of saliva on enamel and dentin erosion. J Dent. 27: 333-339, 1999.

Hannig C, Hamkens A, Becker K, Attin R, Attin T. Erosive effects of different acids on bovine enamel: release of calcium and phosphate in vitro. Arch Oral Biol. 50:541-52, 2005.

Hannig M, Balz M. Protective properties of salivary pellicles from two different intraoral sites on enamel erosion. Caries Res. 35:142-8, 2001.

Hara A, Turssi C, Teixeira E, Serra M, Cury J . Abrasive wear on eroded root dentine after different periods of exposure to saliva in situ. Eur J Oral Sci. 111: 423-427, 2003.

Hara A, Lussi A, Zero D. Biological factors. Mongr Oral Science. 20: 88-99, 2005.

Hara A, Kelly, González-Cabezas C, Eckert G Barlow A, Mason S, Zero D. Influence of Fluoride Availability of Dentifrices on Eroded Enamel Remineralization in situ. Caries Res. 43:57–63, 2009.

Hara AT, Zero DT. The potential of saliva in protecting against dental erosion. Monogr Oral Sci. 25:197-205, 2014.

Jarvinen V, Rytomaa I, Heinonen O. Risk factors in dental erosion. JDR. 70; 942, 1991.

Karlinsey R, Mackey A, Walker E, Frederick K and Fowler C. In vitro evaluation of eroded enamel treated with fluoride and a prospective tricalcium phosphate agent. Journal of Dentistry and Oral Hygiene Vol. 1(4): 052-058, October, 2009.

Kanzow P, Wegehaupt FJ, Attin T, Wiegand A. Etiology and pathogenesis of dental erosion. Quintessence Int. Apr; 47(4):275-8, 2016.

Lagerweij M, Buchalla W, Kohnke S,. Becker K, Lennon Á.M., Attin T. Prevention of Erosion and Abrasion by a High Fluoride Concentration Gel Applied at High Frequencies. Caries Res. 40:148-153, 2006.

Larsen MJ. Prevention by means of fluoride of enamel erosion as caused by soft drinks and orange juice. Caries Res. May-Jun 35(3): 229-34, 2001.

Lussi A, Jäggi T, Schärer S. The influence of different factors on in vitro enamel erosion. Caries Res. 27: 387-393, 1993.

Lussi A, Jaeggi T, Gerber C, Megert B. Effect of amine/sodium fluoride rinsing on toothbrush abrasion of softened enamel in situ. Caries Res. 38: 567-571, 2004.

Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res. 38: 34-44, 2004.

Lussi A. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Mongr Oral Science. 20: 1-8, 2006.

Lussi A, Hellwig E, Ganss C. Buonocore Memorial Lecture. Dental erosion. Operative Dentistry. 34-3: 251-262, 2009

Lussi A. Dental Erosion: Novel remineralizing agents in prevention or repair. Adv. Dent. Res. 21; 13, 2009.

Mathews M, Amaechi BT, Ramalingan K, Cchuana R, Chedjieu P, Mackey A, Karlinsey R. In Situ remineralization of eroded enamel lesions by NaF rinses. Arch Oral Bio. 57 525-530, 2012.

Navazesh M, Satish K. Measuring salivary flow. Challenges and opportunities. JADA, Vol 139. May: 35s-39s, 2008.

Nehme M. Jeffery P, Mason S, Lippert F, Zero, Hara A. Erosion remineralization efficacy of gel-to-foam fluoride toothpastes in situ: A randomized controlled trial. Caries Res. 50:62-70, 2016.

Niklander S, Veas L, Barrera C., Fuentes F, Chiapinni G, Marshall M. Risk factor hyposalivation and impact of xerostomia on oral health related quality of life. Braz Oral Res. 31: 35s-39s, 2017.

Piangprach T, Hengtrakool C, Kukiattrakoon B, Kedjarune-Leggat U. The effect of salivary factors on Dental Erosion on various Age groups and tooth surfaces. J Am Dent Assoc. 140:1137-1143, 2009.

Pedroso C, Messias D, Corona S, Serra M. Variability of using enamel and dentin from bovine origin as a substitute for human counterparts in an intraoral erosion model. Braz Dent J.21(4): 332-336, 2010.

Percival R, Challacombe S, Marsh PD. Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. J Dent Res; 73: 1416, 1994.

Poggio C, Lombardini M, Dagna A, Chiesa M, Bianchi S. Protective effect on enamel demineralization of a CPP–ACP paste: an AFM in vitro study. J Dent 37: 949-954, 2009.

Rehder, Pedroso C, Campos M. Erosion-like lesions progression in human and bovine enamel. Int J Dent. Recife. 9(1): 16-20, jan/mar, 2010.

Ren F, Zhao Q, Malmstrom H, Barnes V, Xu T. Assessing fluoride treatment and resistance of dental enamel to soft drink erosion in vitro: Applications of focus variation 3D scanning microscopy and stylus profilometry. J Dent. 37:167-176, 2009.

Reynolds E. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res. 76; 1587-159, 1997.

Rios D, Magalahaes AC, Polo RO, Wiegand A, Attin T, Buzalaf MA. The efficacy of highly concentrated fluoride dentifrice on bovine enamel subjected to erosion and abrasion. J Am Dent Assoc. 139:1652-6, 2008.

Salas MM, Nascimento GG, Vargas-Ferreira F, Tarquinio SB, Huysmans MC, Demarco FF. Diet influenced tooth erosion prevalence in children and adolescents: results of a meta-analysis and meta-regression. J Dent. Aug;43(8):865-75, 2015.

Scaramucci T, Borges AB, Lippert F, Zero DT, Hara AT. In vitro effect of calcium-containing prescription-strength fluoride toothpastes on bovine enamel erosion under hyposalivation-simulating conditions. Am J Dent. Feb 28(1):18-22, 2015.

Screenby LM, Valdini A. Xerostomia: a neglected symptoms. Arch Intern Med. 147: 1333-7, 1987.

Schlueter N, Tveit AB. Prevalence of erosive tooth wear in risk groups. Monogr Oral Sci. 25:74-98, 2014.

Shellis et al. Methodology and models in erosion. Caries Res. 45(suppl 1): 69–77, 2011.

Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: an in situ study. Arch Oral Biol. 55:541–544, 2010.

Thanabodi Piangprach, Chanothai Hengtrakool, Ureporn Kedjarune-Leggat. The effect of salivary factors on dental erosion in various age groups and tooth surfaces. J Am Dent Assoc. 140(9): 1137-43, 2009.

Tahmassebi JF, Duggal MS, Malik-Kotru G, Curzon MEJ. Soft drinks and dental health: a review of the current literature. J Dent. 34(1): 2–11, 2006.

Tan EC, Lexomboon D, Sandborgh-Englund G, Haasum Y, Johnell K.: Medications that cause dry mouth as an adverse effect in older people: A systematic review and metanalysis. J Am Geriatr Soc. Oct 26, 2017.

Thakar PJ, Badakar CM, Hugar SM, Hallikerimah S, Patel PM, Shah P. An in vitro comparison of casein phosphopeptide-amorphous calcium phosphate paste, casein phosphopeptide-amorphous calcium phosphate paste with fluoride and casein phosphopeptide-amorphous calcium phosphate varnish on the inhibition of demineralization and promotion of remineralization of enamel. J Indian Soc Pedod Prev Dent. Oct-Dec; 35(4): 312-318, 2017.

Turner MD. Hyposalivation and xerostomia: Etiology, complications and medical management. Dent Clin North AM. April; 60(2): 435-43, 2016.

Sever V, Klaric, E, Tarle Z. Accounting for measurement reliability to improve the quality of inference in dental microhardness research: a worked example. Clin Oral Invest. Jul; 20(6): 1143-9, 2016.

Stephan RM, Miller BF. A quantitative method for evaluating physical and chemical agents which modify production of acids in bacterial plaques on human teeth. J Dent Res. 1943;22:45–51.

Vieira A, Ruben J, Huysmans M. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res. 39: 371-379, 2005.

Vukosavljevic D, Custodio W, Buzalaf M. Hara A, Siqueira W. Acquired pellicle as a modulator for dental erosion. Arch Oral Bio, 2014.

West NX, Maxwell A, Hughes JA, Parker DM, Newcombe RG, Addy M. A method to measure clinical erosion: the effect of orange juice consumption on erosion of enamel. J Dent. 26, 329-335, 1998.

West NX, Davis M, Amaechi BT In vitro and in situ erosion models for evaluating tooth substance loss. Caries Res 2011. 45(suppl1): 43-52 2011.

Wegehaupt FJ, Taubock TT, Stillhard A, Schmidlin PR, Attin T. Influence of extra- and intra-oral application of CPP-ACP and fluoride on re-hardening of eroded enamel. Acta Odontol Scand. May;70(3):177-83. 2012.

Zero DT, Fu J, Anne KM, Cassata S, McCormack SM, Gwinner LM. An improved intra-oral enamel demineralization test model for the study of dental caries. J Dent Res. 71. 871-878, 1992.

Zero DT, Fu J, Scott-Anne K, Proskin H. Evaluation of fluoride dentifrices using a short-term intraoral remineralization model. J Dent Res. 73 (Spec Iss), 272,1994.

Zero DT: Etiology of dental erosion - extrinsic factors. Eur J Oral Sci. 104, 162-177,1996.

Zero DT, Barillas I, Hayes AL, Fu J, Li H. Evaluation of an intraoral model for the study of dental erosion. Caries Res. 32, 312, Abst. 132, 1998.

Zero DT, Cavaretta Siegel G, Fu J, Li H. Effect of pyrophosphate on fluoride enhanced remineralization after an erosive challenge. Caries Res. 34, 344, Abst. 105, 2000.

Zero DT, Brennan M, Daniels, T, Papas A, Stewart C., Pinto A., Al Hashimi I, Navazesh M, Rhodus N, Sciubba J, Singh M, Wu A., Franysie J, Fox. Lawrence,

Cohen S, Vivino F, Hammit K. Clinical Practice guidelines for oral management of Sjögrens disease: Dental Caries prevention. JADA. Vol 147, Issue 4. April: 295-305, 2016.

APENDICES

APPENDIX A

Copy of IRB

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Initial Review (Response to Modifications)

November 8, 2010

Adriana Semprum-Clavier, DDS
Restorative Dentistry
801 S Paulina
304 DENT, M/C 555
Chicago, IL 60612
Phone: (312) 355-4856 / Fax: (312) 996-3535

RE: Protocol # 2010-0597
"The Effect of Remineralizing Agents on Dental Erosion in a Reduced Salivary Flow Population: An in situ model."

Dear Dr. Semprum-Clavier:

Your Initial Review (Response to Modifications) was reviewed and approved by members of IRB #1 under the Expedited review process on October 28, 2010. You may now begin your research

Please note the following information about your approved research protocol:

Protocol Approval Period:	October 28, 2010 - July 20, 2011
Approved Subject Enrollment #:	30
Performance Sites:	UIC, University of Texas, Sjogren's Syndrome Foundation
Sponsor:	National Institute of Dental and Craniofacial Research
PAF#:	2007-01020
Grant/Contract No:	5T32DE018381-02
Grant/Contract Title:	Multidisciplinary Oral Science Training Program

Research Protocol:

a) The effect of remineralizing agents on dental erosion in a reduced salivary flow population: An in situ model. Version #2, 08/31/10

Recruitment Materials:

a) Flyer 1; Version 3, 10/11/10
b) Flyer 2; Version 3, 10/11/10
c) Recruitment Email; Version 3, 10/11/10
d) Email Response; Version 3, 10/11/10

APPENDIX B

Recruitment material

1) Patient letter:

Patient Name
Address 1
Address 2

Re: A Research Study You May Be Interested In

Dear Mr/s. Name:

A researcher at UIC, Dr. Adriana Semprum is studying how two different toothpastes help to recover minerals lost from your teeth due to exposure to acidic drinks.

A review of your medical records suggests you might be eligible to participate in this study. Participation will involve receiving an ortho bracket with a little block of cow tooth on one of your *back teeth and* applying special toothpaste at home for two weeks. Study participation will last for a total of 6 weeks since you will try two different toothpastes with a week in between treatments, and will involve at least 5 visits of about an hour each to our research clinics at UIC College of Dentistry.

If you may be interested in participating in this study or have questions, please call Dr. Adriana Semprum at (312) 355-4856. No one will call you about the study unless you call them first.

Participating in research is voluntary. It won't affect your treatment at UIC, If you decide not to call about the study or decide not to participate.

Sincerely,

[*Name of treating doctor*]

APPENDIX B (continued)

2) Clinical Manager Letter

[UIC Dental Clinic Manager's Name]
Address 1
Address 2

Re: Research Study Subject Recruitment

Dear [Dental Clinic Manager's Name]:

My name is Dr. Adriana Semprum and I work in the Department of Restorative Dentistry. I am writing you to ask you for your assistance with subject recruitment for my research study entitled "The Effect of Remineralizing agents on Dental Erosion in a Reduced Salivary Flow Population: An in situ Model," IRB #2010-____. In this study, we will investigate the way eroded tooth enamel incorporates minerals in response to the use of two different toothpastes. The study will be conducted using two groups of subjects; control subjects who have normal salivary flow and subjects who have decreased salivary flow (chronic dry mouth). Because of the specific eligibility criteria for patients with dry mouth, your assistance would be appreciated in recruiting this group.

To be eligible, these subjects must be: 18-64 years of age, have at least 20 teeth (including bridges), have no active caries or periodontal disease, and have either normal salivary flow or complaint of chronic dry mouth. If you have any patients that fit these criteria, I would appreciate it if you could provide them with a copy of my recruitment flyer (see attached.)

Please do not hesitate to contact me at (312) 355-4856 or asemprum@uic.edu if you have any questions.

Thank you for your time and any assistance that you can provide.

Sincerely,

Adriana Semprum, DDS

APPENDIX B (continued)

3) Recruitment Flyer

Principle Investigator: Dr. Adriana Semprum
Department of Restorative Dentistry
801 S Paulina St, Chicago, IL 60612
Research Protocol Title: The effect of remineralizing agents on dental erosion in a reduced salivary flow population: An in situ model
IRB Protocol Number:

RESEARCH SUBJECTS WANTED

This project seeks to investigate the way tooth enamel incorporates minerals in response to the use of different toothpastes. The effects will be compared in subjects with normal salivary flow versus subjects with reduced salivary flow.

We are looking to enroll subjects who are:

- Male or female, 18-64 years of age
- Have at least 20 teeth. Subjects may have fixed bridges replacing missing teeth.
- No current tooth decay, cavities, or periodontal disease
- Have normal salivary flow or reduced salivary flow (chronic dry mouth)

Participants will be asked to come to the UIC College of Dentistry 5 times over the next 6 weeks to:

- Complete a set of questionnaires
- Receive a brief oral examination
- Perform two Salivary Flow Tests
- Have an ortho bracket cemented onto a tooth. The bracket will contain a sterilized piece of enamel derived from cow's teeth.
- Brush with different toothpastes
- Provide two saliva samples

Participants who complete the entire study will be compensated $300 for their time.

For details, please contact Meggan Keller at (312) 996-7749 or email mkelle3@uic.edu.

APPENDIX B (continued)

4) Sjogren Syndrome Foundation Support Letter

We are fortunate when a research center chooses to focus on finding better ways to treat dry mouth and its many complications. Your involvement in these research projects helps to uncover breakthroughs that might help dry mouth patients worldwide.

Enclosed with this letter is a flyer on a clinical research study going on in your area. Your participation in this study may help ensure its success, and I hope you will take a moment to consider it.

For more information and to see if you are eligible to participate, please refer to the enclosed flyer and contact the trial coordinator. If you have questions regarding the project, please contact the number listed in the flyer and not the Foundation Office.

On behalf of the millions of dry mouth sufferers in this country, I thank you for taking time to consider participating in this research study.

Sincerely,

Steven Taylor
Chief Executive Officer

Please Note: You received this notice because of your participation and/or interest in the Sjögren's Syndrome Foundation. The SSF sends this research notice for information only. It does not represent an endorsement of this study but only makes you aware of this research project for your participation if you choose.

APPENDIX C

Subject Accountability Form

Subject ID # _____Slab #_____

Visit 1:
Screening Date:
_____
Unstimulated Salivary Flow Test ☐Yes ☐No
Stimulated Salivary Flow Test ☐Yes ☐No
Colgate toothpaste given ☐Yes ☐No
Group ☐Dry mouth ☐Normal

Visits 2-3:
Bracket cementation Date:
_____
NO Treatment toothpaste given
Bracket decementation Date_____
Unstimulated salivary flow rate: _____
Visits 4-5
Bracket cementation Date:
_____
Treatment toothpaste given ☐Yes ☐No
Toothpaste Code:_____
Bracket decementation Date_____
Treatment toothpaste and Subject Diary returned? ☐Yes ☐No
Stimulated salivary flow rate: _____
Visits 6-7:
Bracket cementation Date:
_____
Treatment toothpaste given ☐Yes ☐No Toothpaste Code:_____
Bracket decementation Date_____
Treatment toothpaste returned? ☐Yes ☐No

VITA

Adriana Semprum-Clavier

EDUCATION

2009-present **University of Illinois at Chicago College of Dentistry and School of Public Health, Chicago, IL**
Trainee for the Master in Clinical and Translational Science Program, Chicago, IL

2001-2004 **University of Detroit Mercy School of Dentistry, Detroit, MI.** Doctor of Dental Surgery. Accelerated program for foreign trained dentists (DDS)

1999-2001 **University of Michigan, Ann Arbor, MI**
Post-graduate Program in Restorative Dentistry

1998-1999 **Universidad Central de Venezuela, Caracas, Venezuela**
Post-graduate Program in Oral Pathology

1995-1996 **Department of Health, Nueva Esparta, Venezuela**
Post-graduate Internship in General Dentistry

1990-1995 **Universidad Central de Venezuela, Caracas, Venezuela.** Doctor of Dental Surgery (DDS)

ACADEMIC APPOINMENTS

2013-present University of Illinois at Chicago. College of Dentistry
Department of Restorative Dentistry
Clinical Associate Professor

2013-present University of Illinois at Chicago. College of Dentistry
Department of Restorative Dentistry
Pre-Patient Care Director for Advanced Standing Program

2005-2013 University of Illinois at Chicago. College of Dentistry
Department of Restorative Dentistry

Clinical Assistant Professor

2004-2005 University of Detroit Mercy. School of Dentistry
Department of Restorative Dentistry
Clinical Assistant Professor

2002-2004 University of Detroit Mercy. School of Dentistry
Department of Restorative Dentistry
Course Director in Operative Dentistry

2001-2004 University of Detroit Mercy School of Dentistry
Department of Restorative Dentistry
Adjunct Assistant Professor

2001-2004 University of Michigan School of Dentistry
Department of Cariology, Restorative Sciences and Endodontics
Adjunct lecturer

2000-2001 University of Michigan School of Dentistry
Department of Cariology, Restorative Dentistry and Endodontics
Research Assistant. Dr. Mathilde Peters

PROFESSIONAL EXPERIENCE

2012-present Uptown Dentistry, 80 N. Northwest Highway. Park Ridge, IL 60068.
Associate in General Dentistry

2005-2009 Dazzling Dentistry, Inc. 6941 South Archer Ave, Chicago, IL 60638. Associate Dentist

1996-1999 Unidad de Atencion Medica-Odontologica. Caracas, Venezuela
Associated Dental Office. General Dentist

1997-1999 Mediservicios. Seguros Orinoco. Caracas, Venezuela
General Dentist (part time employee)

1995-1996 Centro Radiodiagnostico Retex 7. Caracas, Venezuela
Assistant Radiology (part time employee)

1992-1997 Dr. Argimiro Hernandez . Caracas, Venezuela

Internship in Oral Surgery

MEMBERSHIPS

1996-present Colegio de Odontologos de Venezuela
1996-present Colegio de Odontologos Metropolitano
2000 present Academy of Operative Dentistry
2000-present International Association of Dental Research
2004-present American Dental Association
2005-present American Dental Educators Association
2005-present Chicago Dental Society
2005-present Hispanic Dental Association

PUBLICATIONS

Semprum A., Hernandez A.
Hepatitis B: Un Reto para la Odontologia (Hepatitis B: A Challenge For Dentistry).
Revista Inspeccion Sanitaria. Organo Oficial del Colegio Nacional de Inspectores de Salud. 1st edition. 1996. Caracas, Venezuela.

EDUCATIONAL MULTIMEDIA

Peters M., Stoffer K., Jimenez M., Semprum A. Self-Assessment Skills in dentistry. University of Michigan and Digident, Ann Arbor, M, I Aug 2001

ABSTRACT

Semprum A., Wagner W.C., Neme A.M.-L , Peters M.C. Effect of laser treatment on pit and fissure sealant microleakage. IADR, Honolulu, HI. March 10-12, 2004.

Semprum A., Almeida J., Peters M. Nor J., Myaki S., Tanji E. The Effect of Caries Removal Techniques on the Ultraestructure of Dentin. Academy of Operative Dentistry. IADR , San Diego, CA. March 03-06, 2002

Peters M., Stoffer K., Mc Lean M., Green T., Jimenez M., Semprum A. Interactive Enhancement of Self-Assessment Skills in dentistry. ADEA TechnoFair an Technology Expo: J Dental Educ 65 (2) 2001: 152-153

TABLE CLINIC

Semprum A., Almeida J., Peters M. Nor J, Myaki S, Tanji E. Micromorphology of the Dentin Surface Following Caries Removal with Carisolv or Laser. Academy of Operative Dentistry. Chicago, IL. February 22-24, 2002

Effect of Dentin Conditioner in Three Different Hybrid Glass ionomer Cements. Chicago IL. Academy of Operative Dentistry. February 22-24 , 2001

OTHER ACTIVITIES

-Organizer and presenter of the annual meeting for the Regional Central Caries Management by Risk assessment (CaMBRA) coalition February 2008- 2018
-Advisor for the Hispanic Dental Student Association at University of Illinois at Chicago (2006-present)
-Guest speaker at: -AGD conference in Elk Grove. IL. “Updates in Adhesive Dentistry” May 5th 2007.
-Odontographic Society of Chicago. “Adhesive Dentistry”. February 2009
- Sjögren’s Syndrome Foundation support group in Plainfiled, IL March 2010
- Hispanic Dental Association Chicago Chapter. Chicago, IL Jun 2010
- Better Breather Club at Cook County Hospital, Chicago, IL August 2010