Official Study Title: **Remineralization of Molar Incisor Hypomineralization (MIH) with a Hydroxyapatite Toothpaste – an *in situ* study**

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Remineralization of Molar Incisor Hypomineralization (MIH) with a Hydroxyapatite Toothpaste – an *in situ* study

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A. Null Hypothesis

1. Hypomineralized enamel treated with hydroxyapatite toothpaste would not regain up to 20% mineral density relative to healthy enamel.

2. The effectiveness of hydroxyapatite toothpaste in remineralizing MIH would not be significantly different from that of 1450 ppm fluoride toothpaste.

B. Specific Aims

The objective of this *in situ* clinical study is to determine whether a toothpaste formulation containing hydroxyapatite is effective in promoting remineralization of molar incisor hypomineralization (MIH), in comparison to a toothpaste formulation containing 1450 ppm fluoride. Effectiveness will be declared if the MIH lesion regain up to 20% mineral density relative to healthy enamel.

C. Significance / Background

MIH is a qualitative enamel defect observed on at least one first permanent molar (FPM) with or without affecting permanent maxillary incisors[1]. Clinically, it's diagnosed when a FPM presents with a demarcated opacity, larger than 1 mm, varying in color from white to yellow and even brown[2,3]. Additionally, post-eruptive enamel breakdown, atypically sized, shaped, and extended restorations, or a history of extraction of FPMs in an otherwise sound dentition, are also diagnostic criteria of MIH. The location, asymmetry and varying severity of the defects, distinguishes them from other enamel mineralization defects such as fluorosis[4]. The severity of the affection can be divided into 2 categories, mild and moderate/severe. These categories are differentiated clinically by the presence or absence of enamel breakdown, the degree of hypersensitivity and the esthetic concerns[3]. It is also correlated with reduced levels of mineral density (MD) of the affected enamel to 2.24 g/m^3 in mild cases and 1.93 g/m^3 in moderate/severe cases in comparison to a 2.51 g/m^3 average MD of normal enamel[5].

Hypomineralization can affect both primary and permanent teeth, but the molar-incisor model is the most common with a reported prevalence between 10% to 20%[6,7]. As for their etiology, MIH defects can result from genetic or systemic environmental factors. Perinatal and postnatal factors appear to be the most strongly associated with its occurrence[8]. In fact, premature birth and associated complications, hypoxia, dioxin exposure and other childhood illnesses such as ear or respiratory infections and antibiotic use are implicated in an increased incidence of MIH. If these conditions develop in the early childhood, up to age 3, when the crowns of the FPMs and incisors are

undergoing mineralization, they predispose the child to MIH[3,9]. Consequently, the time of onset, span and severity of the conditions dictate the pattern and intensity of the lesions.

Management of MIH defects is complicated by the penetration of bacteria through the often-exposed dentinal tubules[10]. This process causes subclinical inflammation of the pulp and hypersensitivity that lead to a higher anesthetic threshold and negligence of oral hygiene by the child subsequently increasing the need for dental treatment[11]. Additionally, esthetic and functional concerns are frequent in these patients and are associated with a lower oral health related quality of life[12]. The treatment of teeth with hypomineralization defects is twofold, preventive and/or interventional. Therapeutic interventions must take place when the teeth display severe defects, enamel breakdown, decay or signs of pulpal inflammation/infection[13]. This approach includes restoration with glass ionomer, composite resin or preformed metallic crowns for posterior teeth and micro-abrasion with resin infiltration and composite restoration for anterior teeth and extraction with space management for non-restorable teeth[3,14]. However, when MIH diagnosis is established early, the prevention of the progression of the defect is recommended by using sealants and promoting remineralization. In fact, increasing the mineral content of the enamel to a threshold close to the norm can improve its physical properties, hence improving the esthetic and functional outcomes. Several techniques pertaining to remineralization of MIH lesions have been described in the literature including the use of low-level laser therapy or the application of pastes and varnishes containing arginine, fluoride (with or without tricalcium phosphate), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) or casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP)[13]. Toothpastes containing nanohydroxyapatite (HAP) were shown to have comparable effects to fluoride toothpastes in terms of remineralization of incipient carious lesions and prevention of decay, while excluding the risk of fluorosis[15]. The synthesized particles of HAP are structurally similar to the crystals found in enamel and dentin, have analogous properties and can readily penetrate enamel surface[16]. However, their use as a mean of increasing mineral density in MIH defects has not been investigated. Thus, the objective of this study is to determine the effectiveness of a toothpaste containing HAP in promoting remineralization of teeth with MIH defects.

D. Experimental Design

Production of Samples

30 freshly extracted permanent teeth with MIH will be collected from the pediatric clinics of the University of Texas Health San Antonio (UTHSA). These teeth were extracted as part of the patient's standard of care and

donation will be done based on the patients willing ness. If the patient wishes to donate the teeth, they will be placed in a container within the pediatric dental clinics and will be donated without in identifiable information attached to them. The teeth will be cleaned of debris/stains and examined with a transilluminator. Teeth with MIH but free of caries or other malformations will be selected and cleaned with pumice to remove the pellicle. Using a water-cooled diamond wire saw, 3 blocks, two from MIH affected area and one from a healthy area will be produced from each tooth, with each block measuring approximately 2 mm length x 2 mm width x 1 mm thickness. The cut will take place so that the different layers from the outer surface to the inner surface present in each block are: outer enamel surface, enamel, dentino-enamel junction (DEJ) and dentin.

Measurement of baseline mineral density of the tooth blocks

Micro computed X-ray tomography (μ-CT) is a high resolution, non-destructive radiographic method of analysis that generates qualitative images and quantitative analysis of the mineral density (MD) in tooth samples. For this reason, images will be collected from the samples to measure the mineral density of the MIH enamel lesions before (MDb) and after (MDt) remineralization treatment as well as the mineral density (MDs) of healthy enamel samples. The μ-CT scanning and analysis procedure will be performed as described in our previous publications.[17]

Study Population/Participants

Fifteen subjects (8 females, 7 males) will be recruited into the study. The subjects to be included should be 18 to 60 years old in good general health with no known history of allergy to personal care/consumer products. They must have a minimum of 20 natural uncrowned teeth (excluding third molars), no active unrestored cavities and a normal salivary flow rate (stimulated and unstimulated flow of ≥0.7 ml/min and ≥0.2 ml/min respectively) ascertained from a preliminary sialometry test. They also should give written informed consent, be available throughout entire study, and be willing to wear intra-oral appliance 24 hours per day and use only assigned products for oral hygiene throughout the duration of the study.

The exclusion criteria are having advanced periodontal disease, a medical condition that requires premedication prior to dental visits/procedures, an impaired salivary function, wearing an orthodontic retainer(s), not having enough teeth to secure the oral appliance, having diseases of the soft or hard oral tissues, using drugs that can affect salivary flow, using antibiotics one month prior to or during this study, participating in another clinical study one week prior to the start of the washout period or during this study period, using tobacco products, having an allergic

history to common toothpaste ingredients, and having a compromised immune system (HIV, AIDS, immuno-suppressive drug therapy) as determined by review of medical history

Recruitment of Study Subjects

This is a double-blind, randomized, crossover, single center, controlled clinical trial. The study will be blinded to both the study team and the subjects. Recruitment of potential subjects will be from the local San Antonio area. The subjects will be identified with code numbers (CP01 to CP15). The approval of the UTHSA IRB will be obtained. This study will be conducted in compliance with International Conference on Harmonization (ICH) Good Clinical Practice Guidelines. At the screening visit, each subject will complete a medical / dental history and read and sign an informed consent form. Following consent, a visual oral health exam will be performed by a licensed dentist. Subjects will be enrolled based on all the inclusion and exclusion criteria and other screening evaluations such as preliminary sialometry, and then randomly assigned to one of two treatment sequences.

Construction of the *in-situ* appliance

The 3 blocks from each tooth will be used, as follows**:** two blocks with MIH lesions will be used for remineralization assessment and one healthy tooth block will serve as control for the MD of healthy enamel. The MIH-bearing blocks will be covered with polyester gauze (Bard Peripheral Vascular, Inc., USA), which will facilitate plaque retention on the surface of the tooth blocks on intra-oral exposure. Following recruitment, an impression of the subject's lower dentition will be taken using an alginate impression material. A dental technician will fabricate a lower removable intra-oral appliance. Each of the MIH-lesion-bearing blocks will be mounted on each side within the acrylic portion of the removable appliance, using Intermediate Restorative Material (IRM) cement (fluoride–free). All appliances are sterilized with ethylene oxide prior to delivery to the subject.

Test Product Description and Labeling

The toothpastes to be used for treatments are 10% HAP toothpaste, 1450 ppm NaF fluoride toothpaste, and non-fluoride toothpaste (washout). The 2 test products will be coded by the manufacturing/packaging company, who will retain the code until the completion of the study and data interpretation. The experiment will consist of two distinct treatment phases, Phase 1 and Phase 2, during which subjects will be exposed to one of the 2 products in a randomized crossover design, with each phase lasting for 2 weeks.

Study Procedure and Patient Instructions

Prior to each 2-week treatment phase, subjects will complete a 1-week washout period. This period allows for attenuation of any residual effect of the subject's previously used toothpaste. During this period, no appliance will be worn, and subjects will be using only the provided washout toothpaste and a soft-bristled toothbrush twice daily. Following the washout period, the intra-oral appliance will be fitted to each subject by a qualified dentist. Immediately after fitting of the first appliance (on day 1 of the first treatment phase), each subject will receive a soft bristled manual toothbrush for use throughout the duration of the study and a toothpaste according to the treatment phase. They will make the first use of the test product under the supervision of the study coordinator. For the remainder of the study, subjects will complete the procedure at home and as instructed.

The subjects will be instructed to brush their teeth with the appliance in the mouth, two times daily, for 3 minutes on each brushing episode, in the morning after breakfast and last thing before bed, then rinsing with 10 ml of water. In dispensing the toothpaste onto the toothbrush, the subjects should fill the toothbrush surface from end to end but no more than one ribbon of toothpaste. The subjects will be advised not to brush directly on the blocks but rather to brush around the blocks to prevent disruption of the plaque. Subjects should neither eat nor take any drink for at least 30 minutes after brushing, they will be also instructed to remove the appliance while eating. A timer and measuring cup will be provided to each subject. To monitor product usage and compliance, a diary will be provided to each subject and checked at every visit, to record the number of tooth-brushings performed each day and the time it was done. Further, subjects will be instructed to return the remaining washout or test toothpaste after each treatment phase. The weight of toothpaste will be measured before and after each treatment phase. Over the study period, all subjects are to maintain their normal dietary habits.

On day 15, the subject, without using the product that morning, will arrive at the clinic, and the tooth block will be harvested and sent to the laboratory for μCT analysis. Then the subject will be given washout toothpaste and a soft-bristle toothbrush to undergo another 7-day washout period without an appliance. After completion of the second washout period, subjects will return to the clinic, and the appliance, with another MIH-bearing tooth blocks mounted, will be fitted to the subject for the phase 2 treatment period. This procedure will then be repeated until the 2-week treatment phase is completed, and each subject has gone through the two arms of the study.

Upon completion of the treatment phases, the post-remineralization mineral density (MDt) of all the MIH-lesion-bearing blocks exposed intra-orally for remineralization will be assessed with μCT. For the lesion-bearing blocks,

this process will yield the following information: the pre-test (MDb) and post-test (MDt) mineral density of the lesions and their associated images, while for the healthy tooth blocks, the MDs and the μCT images.

At every visit, the dental examiner will visually examine the soft and hard tissues of the oral cavity and peri-oral area using a dental light and dental mirror. Additionally, subjects will be asked about and examined for any adverse events. Subjects may call and request a visit for any concern of potential adverse event.

Data Handling

Using the μCT images, the pattern and the extent of remineralization produced within each lesion by each treatment product will be examined and described. This will be clearly shown by comparing the pre- and post-test images side-by-side. For all calculations, the absolute MD will be measured and used. For the remineralization blocks, the values of the MDb and MDt for each test product will be compared using paired t-test to determine any significant change (remineralization) made by the test product (intra-group comparison). However, to make comparisons between the two products (intergroup comparison), percentage change in MD calculated relative to the control will be determined for each test product as such: % Change in MD (%ΔMD) = [(MDb – MDt)/MDb] x 100

The percentage MD recovery following remineralization will be calculated as follows:

% MD recovery (%MDR) = [(B-T)/(B-S)] x 100, with S = mean MDs of healthy enamel blocks, B = mean MDb of MIH enamel blocks before remineralization, and T = mean MDt of MIH enamel blocks after intra-oral exposure.

Sample Size Calculation

The power analysis and sample size calculation were performed using nQuery Advisor software (Statistical Solutions, Cork, Ireland). The sample size for this study is based on the primary efficacy parameter, % MDR following 2 weeks of treatment. A sufficient number of subjects will be screened to randomize approximately 15 subjects with the intention that approximately 15 subjects complete all study treatments and be evaluable for the efficacy analysis. With these conditions met, the study will have 80% power at the 5% significance level, using two-sided testing, to detect a mean treatment difference in %MDR of approximately 8.1% assuming a within subject standard deviation of approximately 12.6%. In the absence of available 2 week data from previous mineral density studies, the within subject standard deviation has been estimated using 14-day post-treatment data from a previous results obtained by this group in a demineralization and remineralization study using HAP toothpaste[15]. In that study the mean % change in mineral loss was equal to 30.3 with a standard deviation equal to 16.3.

Statistical Analysis

After assumptions of normality and homogeneity of variances have been verified by normal probability plots, comparisons of the mean values of MD, %ΔMD and %MDR between the two treatment groups as well as the intra-group comparisons will be performed using the paired Student's t-test (STATA version 10.0, StataCorp LP, USA) at a significance level of 5%. The primary endpoints of the present study are expected to be enhanced remineralization of MIH lesion.

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