THE EFFECT OF ACID ETCHING ON REMINERALIZATION OF INCIPIENT CARIES LESIONS: A MICRO-CT STUDY

by

Hanin E.Yeslam

Submitted to the Graduate Faculty of the School of Dentistry in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University School of Dentistry, 2009.

Thesis accepted by the faculty of the Department of Restorative Dentistry, Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

_____

Carlos Gonzalez-Cabezas

_____

Melvin Lund

_____

Tien-Min Gabriel Chu

_____

Masatoshi Ando
Chair of the Research Committee

_____

Michael Cochran
Program Director

Date_____

ACKNOWLEDGMENTS

I would like to convey my deepest appreciation and thanks to my research committee, Drs. Michael Cochran, Masatoshi Ando, Carlos Gonzalez-Cabezas, Melvin Lund, and Tien-Min Gabriel Chu for their professionalism and support.

I will always be deeply grateful to my mentor, Dr. Masatoshi Ando, for his guidance, expertise, and thoughtful criticism, which allowed me to appreciate scientific curiosity and integrity.

I would like to extend my immense gratitude to the lab technicians who helped me throughout the course of my work. I would like to dedicate a special thanks to Jennifer for her great help and guidance in the lab.

I would like to express my sincere and heartfelt thanks to my mother and father for their continuous support, love, and encouragement. Without their prayers and support, my professional growth would not have been possible.

Finally, I am deeply grateful to my husband for his support and encouragement. Thanks to my wonderful little daughter, Joory, for the sacrifice that she innocently made during the long hours of study and lab work. Those are the people for whom I live, and this thesis is dedicated to them.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Since the introduction of fluoride in the dental community for caries prevention in the 1940s,[1] the prevalence of arrested incipient carries lesions has increased.[2] However, the depth of remineralization associated with the application of topical fluoride solution has been studied extensively with controversial results. One of the documented effects of lesion arrestment induced by fluoride application is the preferential deposition of minerals in the surface layer of the enamel. Although the subsequent formation of the hypermineralized, superficial layer of enamel causes arrestment of the caries lesion, it hinders the full remineralization of the body of the lesion.[3-5]

It has been hypothesized in previous studies that removing the superficial hypermineralized layer would increase the mineral diffusion into deeper layers in the lesion. Etching of an enamel caries lesion prior to remineralization has been shown to enhance the remineralization process by increasing the surface area of the enamel and by producing a certain degree of surface porosity allowing further mineral diffusion into the lesion.[4, 6, 7] This enhancement effect for remineralization has been found to reach a plateau after a period of time. To overcome this problem, additional measures have been suggested, including the administration of a second etch to the enamel surface.[4]

Preferential deposition of minerals in the surface layer is considered to be one of the reasons hindering remineralization of the porous body in opaque, incipient caries lesions.[4, 8] Therefore, a simple technique to enhance the fluoride's remineralization effect and to increase the porosity of these lesions would be advantageous for the preventive dental clinics providing caries arrestment regimens.

The primary objective of this *in-vitro* study was to investigate whether the application of an additional acid etching to the surface layer during remineralization would enhance the remineralization process. A secondary objective was to evaluate the use of microcomputed tomography (μ-CT) to assess the mineral density of caries lesions. The μ-CT is the most recently developed non-destructive method and can be used to quantify mineral content in hard tissues. It allows the study of very thin sections of the tissues with more consistency by alleviating the probability of mechanical cutting error. Further investigations of the μ-CT are needed to increase the validity of its results.

NULL HYPOTHESIS

There is no enhancement of mineral uptake in the caries lesion by the application of an additional acid-etching treatment to the surface layer during the remineralization process.

ALTERNATIVE HYPOTHESIS

There is an enhancement of mineral uptake in the caries lesion by the application of an additional acid-etching treatment to the surface layer during the remineralization process.

REVIEW OF LITERATURE

ENAMEL STRUCTURE

Enamel is the most mineralized tissue in the body. Human enamel consists of a mineral phase that occupies about 96 percent by weight and approximately 87 percent by volume.[9] It contains millions of rods that look like keyholes running from the dentinoenamel junction (DEJ) to the enamel surface when viewed cross-sectionally under the microscope.[9] The rods are separated by a protein matrix. The mineral phase is essentially composed of polycrystals similar in structure to hydroxyapatite crystals $Ca_{10}(PO_4)_6(OH)_2$ with many impurities, mainly carbonate, which replaces phosphate, and which increases solubility.[10, 11] The cross-sectional shape and arrangement of these crystals differ between enamel layers. In the surface layer, the crystals show size and shape variability while being densely packed, resembling the appearance of a "stone wall." On the other hand, hexagonal crystals in the middle and deep layers are somehow smaller with gaps in between them.[12]

When teeth erupt, they are complete from an anatomic perspective, but not complete in regard to their crystallographic structure.[13] Following eruption, a life-long, continuous maturation process occurs, ranging from daily replacement of ions to slow repair of extensive, subsurface lesions caused by long-term negative caries balance.[13, 14]

DENTAL CARIES

Without comprehensive knowledge of the caries process, one is most likely to envision the process of the carious lesion development as a continuous phenomenon leading to the everlasting mineral loss causing the formation of a clinically detectable cavitation. The process of dental caries is better understood nowadays, although there are still some details yet to be determined.[15]

Dental caries is a transmissible bacterial disease caused mainly by two groups of bacteria, namely the *mutans streptococci* and *lactobacilli* species that ferment the ingested carbohydrates to produce organic acids resulting in tooth-mineral dissolution.[13, 15-17] The caries process is a dynamic continuum that results from numerous cycles of demineralization and remineralization,[13, 18] where the balance between the two processes determines the eventual outcome of dental caries and the site where the caries lesion develops.[15] *In-vitro* caries models have been developed as early as 1982. Their development allowed the discovery of processes involved in caries in much shorter time while new intervention treatments could be tested and modified.[15, 18, 19]

The earliest clinically detectable sign of dental caries in the mouth is the incipient enamel lesion known as a white spot, which becomes detectable radiographically as the lesion progresses into the enamel and dentin. At this stage, before cavitation, therapeutic measures can be applied to reverse or arrest the lesion.[20]

Histologically, incipient caries lesions consist of zones that can be demonstrated with *in-vitro* models as well. These zones are identified histologically in enamel lesions arranged from the outer surface of the tooth reaching the DEJ:

- Surface zone, with a crystal diameter of 40 mm.

- Body of the lesion, with a crystal diameter of 10 mm.
- Dark zone, with a crystal diameter of 50 mm.
- Translucent zone, with a crystal diameter of 30 mm.

Both the surface and dark zones are remineralization zones. Both the translucent zone and the body of the lesion are demineralization zones.[20, 21]

DEMINERALIZATION

Demineralization is basically the loss of mineral apatite from the enamel.[22, 23] When organic acids, such as lactic and formic acids, are produced by acidogenic bacteria, they diffuse in various directions through the enamel and dentin organic matrix into underlying tissues.[11, 13, 20, 24, 25] The organic matrix accelerates the demineralization process by providing permeable channel networks for acid invasion.[23] When the acid reaches a susceptible site on the crystal surface, minerals dissolve into the surrounding aqueous phase. The first ions to be released are sodium, magnesium, and carbonate, followed by calcium and phosphate, which would act as a buffer to help to limit the damaging effects of the acids.[26] This is the first step of demineralization, which takes place at the atomic level before any clinically visible sign is observed. Critical pH, which is about 5.5 for enamel, is the pH level at which demineralization occurs. If the calcium and phosphate supersaturation levels are restored and aided by fluoride, minerals will diffuse into the tooth and deposit a new, more acid-resistant veneer on the crystal remnants in the non-cavitated lesion.[15]

Since the 1940s, it has been noted that the outermost enamel layer is the most resistant to dissolution.[14] Two mechanisms have been proposed for the formation of this hypermineralized surface layer of incipient lesions. The first is the deposition of fluoride

and other ions from saliva. The other is the outward diffusion of minerals and ions from the subsurface lesion that would be deposited in the surface layer.[14, 27]

REMINERALIZATION

Over time, researchers have been more interested in utilizing various remineralization techniques, and in detecting incipient lesions as early as possible to prevent further cavitations.[28]

Remineralization is the body's natural repair process of enamel rod structure following acidogenic episodes.[29] The basic mechanism of remineralization involves the diffusion of calcium and phosphate from saliva and other topical sources aided by fluoride to build a hypermineralized, acid-resistant, fluoroapatite-like veneer on the existing crystal remnants, which act as remineralization nuclei.[13] This is one crucial mechanism of action of fluoride in the inhibition and reversal of the caries process.[13, 29] Enamel surface is in a dynamic equilibrium with its local, oral environment with a constant movement of ions.[15, 18, 30] Except under unusual circumstances, demineralizing conditions in the mouth are transient. The extent of demineralization relates inversely with the duration of the exposure and frequency of acid attacks.[30] An increased amount of saliva minimizes the effects of acids produced by bacteria. This is attributed to the saliva that washes out cariogenic residues and exhibits a buffering capacity. [13, 14, 30]

Considerable clinical evidence for remineralization can be traced back to 1912 when a physician, Head, demonstrated that teeth underwent cycles of softening and hardening. Fluoride was first noted for its mottling effect on enamel in the 1930s, when various community drinking-water reservoirs were investigated. Fluoride's caries-preventive effect was introduced in the mid 1940s, when the difference in caries

prevalence between communities was correlated with the variation in fluoride concentration in drinking water.[1, 31] Studies by Dean and Parfitt suggested and further investigated the possibility of the use of fluoride for mass-caries control.[1, 32] Remineralization of superficial enamel lesions is demonstrated in both *in-vitro* and *in-vivo* studies confirming the remineralization effectiveness of fluoride.[33] An *in-vivo* study in 2001 reported that the inner enamel and dentin are capable of remineralization. But, the deeper the levels of demineralization, the more slowly remineralization would occur.[28]

Remineralization is studied from two perspectives. The first is the process of filling the enamel defects formed due to demineralizing, acidogenic episodes by mineral deposition. In this case, the demineralization and remineralization magnitude determines whether a lesion would develop or the tooth remains sound. The second is repairing an incipient lesion that has already developed but still could be filled completely or partially with calcium phosphates under suitable remineralizing conditions.[34]

REMINERALIZATION ENHANCEMENT

Since the introduction of fluoride in community water supplies, the prevalence of arrested, incipient carries lesions increased.[2] The major beneficial effect of fluoride is its accelerating effect on the deposition of minerals during the remineralization process. This process involves the preferential deposition of minerals that cause the formation of a hypermineralized surface layer more resistant to acidogenic attacks, thus resulting in arrestment of the incipient caries lesion.[13, 30, 35] The amount of remineralization that is aided by the application of topical high concentration fluoride solution has been studied extensively with resultant controversy regarding the depth of remineralization and

whether complete remineralization of the body of the lesion would occur.[28, 36-38] One of the reasons proposed for incomplete remineralization of the body of the lesion is the preferential deposition of minerals on the surface layer, which decreases porosity and prevents further minerals from reaching deeper lesion areas.[3-5, 39]

A proposed mechanism to overcome this problem and to improve the appearance of such lesions is microabrasion of the enamel surface using hydrochloric acid (HCL) and an abrasive. Although this procedure occasionally improves the appearance of white spot lesions, it results in the removal of considerable amounts of enamel and may cause soft tissue damage by the HCL solution.[8, 40, 41] Another proposed mechanism to enhance incipient carries lesions' remineralization and appearance is acid etching of the enamel surface layer. Phosphoric acid etching would remove the fluoride-rich layer and expose more reactive enamel crystals without affecting the porosity and mineral content of the underlying tissues in the body of the incipient enamel lesion.[8, 42-45] Enamel fluoride uptake is reported to be inversely proportional to its initial fluoride content, and therefore, removal of the surface layer with its higher fluoride concentration would allow for better fluoride uptake.[4, 18, 46] Furthermore, this procedure would not only increase the surface layer porosity but also provide for a larger surface area available for fluoride-mediated remineralization processes to occur.[7]

A more pronounced reduction in lesion depth after remineralization in acid-etched incipient enamel lesions was reported in several studies.[4-6, 42] Flaitz and Hicks reported an increase in the thickness of the surface layer in the remineralized acid-etched group.[6] Al-Khateeb et al. reported an increase in the amount of minerals deposited in the enamel surface and body of the lesions as detected by TMR analysis.[4] In the latter study,

the enhanced remineralization seemed to plateau after several weeks, which suggests the possible need for further etching treatments during the remineralization process.[4]

MINERAL CONTENT MEASUREMENT

Mineral concentration distribution can be measured either directly by chemical analysis of micro-samples or indirectly (e.g. transverse microradiography (TMR). These can also be regarded as either being destructive or non-destructive to the tissue sample. Chemical analysis would involve complete dissolution of the microsample that was obtained by abrasion, acid etching, or microdrilling, providing only the mineral content in the layer that was obtained as a whole.[47] Conventional microscopy methods such as contact microradiography and scanning electron microscopy are usually two-dimensional and involve destruction of the sample. They allow examination of lesions, either before or after the experimental procedures.[48] For examination of sections, transverse microradiography (TMR) is preferred to polarized light microscopy (PLM), mainly due to its direct relation to mineral content.[47] However, both require physical sectioning of the specimens that would cause the loss of some structure between the sections. Accuracy in determining the linear attenuation coefficient depends on the section being aligned parallel with a well-known thickness at the point of measurement.[49] Although TMR requires the preparation of very thin sections, it's still considered the gold standard for determining mineral loss and lesion depth with its higher sensitivity for minute lesion depth progression.[50, 51]

TRANSVERSE MICRORADIOGRAPHY (TMR)

Transverse microradiography is considered to be the gold standard for determining mineral changes in experimental caries lesions against which newly developed caries diagnosis technologies are compared and validated.[50, 51] Ever since its introduction into the dental research field in 1963,[52] TMR has undergone continuing technique advancements that allowed the most detailed quantitative mineral density profile data to be collected. The use of TMR for mineral content analysis has been applied not only post-experimentally, but also for single-section demineralization or remineralization experiments.[50, 51, 53, 54] The use of microradiographs for analysis of enamel thin sections on different occasions proved to be a reproducible and very reliable technique given the standardization of steps applied during the procedure.[51, 54]

TMR requires the preparation of transverse planoparallel thin sections with high precision or lapping in order to preserve the surface structure of the calcified tissues being tested.[49-51, 55] The sections produced for TMR analysis should be thin enough to allow only partial absorption of x-rays in a simple relation to the mineral content. The calculation of the lesion profile is produced by using the mineral contents of sound tissues as reference points.[50, 51]

Following the preparation of thin sections, x-ray absorbance images are produced on photographic plates and their optical densities are converted to mineral content values by calibration with an aluminum stepwedge.[4, 51] Nowadays, a video camera is used for recording the optical density of microradiographs, which allowed the examination of sections on screen monitors.[56] Computer programs have been developed to allow adjustment of the scanned area according to the experimental needs. This has allowed the

use of TMR for measurement of both minute changes in the surface structure and overall changes in the mineral content of the section as a whole.[51, 57]

The largest source of variation between TMR studies arise from the varying definitions of the start lesion at depth zero, which is known as IML. IML definition in studies ranges from a mineral content of 0.0-volume percent, to 20-volume percent, to sometimes the volume percent at the steepest part or the top of the surface layer in the mineral content-depth profile. A smaller source of variation arises from the varying definitions of lesion depth and mineral content for sound tissues.[51]

X-RAY MICROTOMOGRAPHY (μ-CT)

Microcomputed tomography (μ-CT) is a microscopic version of computed tomography that allows non-destructive visualization of the morphological characteristics of teeth and the determination of the mineral content in teeth and bones.[48, 49, 58-63] The major difference between μ-CT systems and medical CT scanners is that the specimen moves while the x-ray source and detector are stationary in the μ-CT units.[49] The μ-CT method has several advantages, including a constant slice thickness that only requires the x-ray beam. This allows the acquisition of much thinner slices than can be acquired with mechanical cutting.[59] Elimination of the mechanical cutting step allows for a much simpler sample preparation, because the procedure only requires the tissues to remain hydrated during the scan period to preserve the sample's physical properties.[49, 64] It also allows accurate measurement of linear attenuation coefficients of mineralized tissues,[49] and real-time monitoring of structural and compositional changes.[49] Currently, μ-CT is used for 3-D rendering and quantification of mineral content in tissues in medicine and dentistry.[49, 62, 65, 66]

X-ray microtomography studies of biological hard tissues can be divided into studies of cortical and cancellous bones and those of teeth. Teeth and cortical bone studies concentrate on the measurement of the degree of mineralization, while cancellous bone studies concentrate on trabecular, morphological measurements.[49] Mineral concentrations in cortical bones can be obtained by converting their linear attenuation coefficients while assuming they're comprised of protein and pure hydroxyapatite, and by using calculated, mass-attenuation coefficients.[67] A volume unit in μ-CT images, the voxel, can be regarded as the elementary volume of bone (EVB), which is the volume of bone composed of mineral, collagen, small blood vessels, cells, water and other organic components.[49] Mean trabecular thickness and trabecular density of cancellous bones can be digitally calculated in three dimensions that allow the generated slices to be constructed in various orientations. Cancellous bone trabecular microstructure can be visualized in any of the three dimensions by using μ-CT as the examination technology.[51, 68, 69]

X-ray microtomography has been increasingly used for studying dental structures, including dentin;[70] *in-vitro* enamel lesion development and caries research;[71] assessing root canal preparation and obturation;[72] root resorption,[73, 74]and enamel.[75] It has been reportedly used for calculation of the elastic modulus of dental materials. In dental materials research, μ-CT is used to provide both qualitative and quantitative data. In order to obtain accurate quantitative mineral content data from dental structures, a reliable means of calibration is needed.[64] External calibration standards or phantoms have been discussed in the literature where commercially available phantoms and custom phantoms were used. Despite the difficulty of mimicking the complexity of dental

structures, phantoms should be constructed from materials that best relate to the examined structure.[64, 76, 77] Accuracy of μ-CT images also depends on the filters used to decrease noise by removing the low-energy soft x-rays that scatter rather than penetrate the object under study efficiently. Several filter materials have been used in the literature, which included varying thicknesses of aluminum, copper, or both.[64, 76, 78]

MATERIALS AND METHODS

STUDY DESIGN

In this *in-vitro* study, 40 (1 mm × 2 mm) sound human enamel specimens were used. The specimens were demineralized in order to form artificial subsurface caries-like lesions. Ten specimens were randomly selected and sectioned for TMR analysis. The remaining specimens were then randomly assigned to three groups each with 10 specimens. Group A was subjected to a pH-cycling remineralization regimen with 1100-ppm sodium fluoride for 20 days. Group B specimens' surfaces were etched with 35-percent phosphoric acid for 30 seconds and then treated as group A. Group C was treated like group B, but with an additional acid- etch application after 10 days of the remineralization regimen. Additionally, mineral volume was assessed by μ-CT before demineralization, and before and after the remineralization treatment. For comparison, TMR was used to measure the mineral content and lesion depth before and after the remineralization treatment for the three groups.

SPECIMEN PREPARATION

The specimens were prepared from extracted sound human teeth, excluding third molars, and the specimens were obtained from oral surgeons and stored in a 0.1-percent thymol solution from the time of extraction. All teeth were obtained in accordance with current human-tissue acquisition regulations. The collection of human teeth for use in dental laboratory research studies was approved by the Indiana University-Purdue University Indianapolis (IUPUI) Institutional Review Board (IRB#0306-64).

Specimens were cut using a water -cooled Isomet low-speed saw into 2 mm × 2 mm specimens. Then, these were divided into 1 mm × 2 mm specimens using the hard tissue microtome (Series 1000 Deluxe hard tissue microtome, Scifab, Lafayette, CO). The cut-enamel blocks were imbedded into epoxy resin (EPO-TEK 301, Epoxy Technology, Inc., Billerica, MA), leaving the top of the 1 mm × 2 mm enamel surface exposed. The exposed enamel surfaces were ground using 1200- and 4000-grit silicon carbide paper in the Struers polishing unit (Struers Inc., Westlake, OH) to remove approximately 50 μm of the surface and thereby remove the hypermineralized enamel surface layer to expose the reactive enamel structure. The specimens were then polished to a higher luster using the polishing pad and diamond polishing liquid (dp suspension, Struers Inc., Westlake, OH) following standard methods (SOP#L012). Then, the polished enamel surface of each specimen was cleaned by sonication in a microliquid solution (2% Micro-90% Liquid Soap, International Product Corp., Burlington, NJ). The surface of each specimen was evaluated for any microcracks or residual acrylic on the enamel surface using a stereomicroscope (X20 magnification) and a suitable light source. Specimens were then stored under humid conditions in closed, labeled containers. The 40 sound enamel specimens were assessed prior to demineralization using μ-CT (SKYSCAN 1172 High Resolution Micro-CT, SKYSCAN, Kontich, Belgium) as described below.

LESION FORMATION

Subsurface lesions were produced in the enamel specimens using a 96-hour immersion at 37˚C in a solution of 0.1-ml lactic acid and 0.2-percent carbopol that has been 50-percent saturated with hydroxyapatite and adjusted to pH 5.0 following standard

methods (SOP#L603). The specimens were then removed from the solution and rinsed in deionized water (DI water). Specimens were finally stored in individually marked vials under humid conditions.

GROUP ASSIGNMENT

Ten specimens were randomly selected and removed for TMR baseline lesion analysis. The remaining 30 enamel specimens were included in the remineralization stage of the experiment. The specimens were randomly assigned to the different experimental groups. The specimens were coded and divided into three treatment groups (10 specimens per group): A, B, and C. Group A (positive control group) was subjected to the remineralization treatment directly after lesion formation. Groups B and C were etched with 35-percent phosphoric acid for 30 seconds and then washed with a stream of deionized water for 30 seconds. Next, the three groups were treated in a remineralization regimen using a remineralization /demineralization pH cycling model. The specimens were exposed to a 50:50 mixture of pooled human saliva and a mineral solution containing 2.2 g/L gastric mucin, 0.381 g/L NaCl, 0.213 g/L $CaCl_2$-$2H_2O$, 0.738 g/L KH_2PO_4, and 1.114 g/L KCL at pH 7.0 and 37˚C. The collection of human saliva for use in dental laboratory research studies was approved by the IUPUI IRB # 0304-58. The specimens were subjected to a four-hour-per-day acid challenge in the lesion-forming solution described previously, and then in fresh 50:50 saliva for one hour, which was changed each day during the acid-challenge period of the treatment regimen. All groups were exposed to four daily fluoride treatments (25 % wt of 1100-ppm fluoride from a NaF toothpaste solution) for 1 minute (Table II). After 10 days of the remineralization/

demineralization cycle, group C was subjected to a second etch with 35-percent phosphoric acid for 30 seconds followed by washing with a stream of deionized water for 30 seconds. After the acid-etching treatment, group C was then returned to the remineralization/demineralization pH cycle model for the remainder of the experiment. The pH cycling remineralization/demineralization regimen was carried out for a total of 20 days.

X-RAY MICROTOMOGRAPHY (μ-CT) MEASUREMENT

Mineral density of the specimens was measured using μ-CT (SKYSCAN 1172 high resolution micro-CT; SKYSCAN, Kontich, Belgium) before lesion formation (sound), after lesion formation, and at the end of the remineralization treatment. Both the specimens and the phantom, which is used as an internal standard for mineral density quantification and μ-CT calibration, were scanned with x-rays generated by a sealed microfocus x-ray tube set at 75 keV and 134 μA current, at 2k-high camera resolution setting. A 0.5-mm thick aluminium filter was placed in front of the detector to remove low energy x-rays below 30 keV. The generated x-ray images were then reconstructed using the NRECON reconstruction software (NReconServer64bit version, SKYSCAN, Kontich, Belgium). Reconstruction of the images was done using a 20-percent ring artifact reduction and a 40-percent beam hardening with a histogram scale from 0.005 to 0.2333096. Cross-sections of the middle portion of each specimen were then used for the μ-CT analysis completed with the CT analysis software (CT An [v.1.9], SKYSCAN, Kontich, Belgium). The volumetric mineral content (VM [μm^3]) of the lesion was determined between 91 and 0 wt%.

TRANSVERSE MICRORADIOGRAPHIC MEASUREMENT

TMR was used to determine the mineral loss (TMR IML) and lesion depth of 100- μm sections obtained from 10 randomly selected specimens at the baseline lesion stage. In addition, at the end of the demineralization and remineralization regimen, one 100-μm section was obtained from each specimen in the three experimental groups for TMR analysis. The enamel sections were obtained by using the hard-tissue microtome (Series 1000 Deluxe hard tissue microtome, Scifab, Lafayette, CO) and then stored in individually marked vials under humid conditions. The enamel sections and the aluminum calibration stepwedge were then mounted and radiographed on a 2"×2" high resolution photographic plate (K1A Photoplates, Microchrome Technology, Inc., San Jose, CA) with Ni-filtered Cu-Kα radiation (Philips x-ray generator, Philips Electronic Instruments Inc., Mahwah, NJ) at 30 mA and 20 kV for 65 minutes. The plates were developed according to the manufacturer's instructions. Two parameters were measured; mineral content loss (TMR IML [% volume mineral × μm]) and lesion depth (LD [μm]) using TMR software (TMR 1.24; Inspektor Research Systems, Amsterdam, The Netherlands).

STATISTICAL ANALYSIS

The μ-CT outcome used to assess remineralization was the percent mineral recovery in 91-0 Wt%, calculated using the formula 100% × (remineralization mineral volume - demineralization mineral volume) / (sound mineral volume - demineralization mineral volume). Comparisons were made among the three groups for differences in remineralization as measured by using the μ-CT for percent mineral recovery in 91-0

wt%. Additional comparisons of TMR lesion depth (μm) and IML (%volume mineral × μm) were performed using one-way analysis of variance (ANOVA). Pair-wise comparisons among the groups were performed using Fisher's Protected Least Significant differences to control the overall significance level at 5 percent. Correlation coefficients and plots were used to assess the correlations among the μ-CT and TMR parameters.

SAMPLE SIZE JUSTIFICATION

A previous pilot study was conducted using the μ-CT and TMR. For μ-CT, after 96 hours demineralization followed by six days remineralization, the mean (SD) percent mineral recovery in 91-0 wt % was 31 (6) and after 12 days remineralization, the mean (SD) was 41 (6). For TMR, after 96 hours demineralization followed by 12 days remineralization , the mean (SD) IML was 2700 (260) %volume mineral × μm, and the mean (SD) lesion depth was 96 (7) μm. To be conservative in the calculations, the standard deviations used in the calculations were 7.5 percent change in 91-0 Wt %, 300 %volume mineral x μm^3 for TMR IML, and 8 μm for TMR lesion depth. With a sample size of 10 per group, the study had an 80-percent power to detect a difference between any two groups of 10 for percent mineral recovery in 91-0 Wt%, assuming a two-sided test at a 5-percent significance level. With a sample size of 10 per group, the study had an 80-percent power to detect a difference between any two groups of 397 %volume mineral × μm for TMR IML and 11 μm for TMR lesion depth.

RESULTS

Table III summarizes the mean and standard error for each outcome by group. The following sections and tables will present more detailed statistical data for the groups and the procedures that took place.

THE μ-CT DATA ANALYSIS RESULTS

The three groups did not have significantly different μ-CT percent mineral recovery from demineralization ($p = 0.82$).

In the sound specimens, it was found that Group A had the highest mineral volume of 91-0 wt% with a mean value of 2.90×10^5 μm³, followed by the Control Demin group with a mean value of 1.64×10^5 μm³, Group B with a mean value of 1.42×10^5 μm³, then Group C with a mean value of 1.04×10^5 μm³ (Table IV).

In the demineralized specimens, it was found that Group A had the highest mineral volume of 91-0 wt% with a mean value of 9.67×10^5 μm³, followed by the Control Demin group with a mean value of 8.32×10^5 μm³, Group B with a mean value of 6.47×10^5 μm³, then Group C with a mean value of 4.34×10^5 μm³ (Table V).

In the remineralized specimens, it was found that Group A had the highest mineral volume of 91-0 wt% with a mean value of 4.67×10^5 μm³, followed by Group B with a mean value of 2.32×10^5 μm³, then Group C with a mean value of 2.28×10^5 μm³ (Table VI).

The μ-CT percent mineral recovery was found highest in Group B with a mean value of 74 percent, followed by Group A with a mean value of 69 percent, then Group C with a mean value of 64 percent (Table VII).

TMR DATA ANALYSIS RESULTS

TMR IML Data

The Control Demin group had the highest IML with a mean value of 1572 %volume mineral × μm, followed by Group B with a mean value of 1180 %volume mineral × μm, then Group C with a mean value of 1157 %volume mineral × μm, then to lesser extent Group A with a mean value of 1012 %volume mineral × μm (Table VIII).

Only the Control Demin group had significantly different TMR IML compared with the other groups ($p < 0.05$ for other groups compared with Demin; $p > 0.30$ for other groups compared with each other).

TMR Lesion Depth Data

In regard to TMR lesion depth, both the Control Demin group and Group A had the deepest lesions with a mean value of 52 μm, followed by Group B with a mean value of 43 μm, then Group C with a mean value of 42 μm (Table IX).

The groups did not have significantly different TMR lesion depth ($p = 0.07$).

TMR and μ-CT Correlation

The correlation between TMR IML and TMR lesion depth was 0.66 ($p < 0.0001$) (Figure 12).

The μ-CT percent mineral recovery from demineralization was not correlated with TMR IML ($r = 0.19$, $p = 0.53$) (Figure 13).

The μ-CT percent mineral recovery from demineralization was not correlated with TMR lesion depth ($r = 0.13$, $p = 0.67$) (Figure 14).

TABLES AND FIGURES

GROUP	DESCRIPTION
Control Demin	Demineralized specimens.
Group A	Specimens gone through remineralization without acid etch.
Group B	Specimens gone through remineralization with acid etching once.
Group C	Specimens gone through remineralization with acid etching twice.

TABLE II

Remineralization/demineralization pH cycling model schedule

TIME	PROCEDURE
8:00 am	Fluoride for 1 min then rinsed for 30 seconds using deionized water (DI water). Then immersed in 50:50 saliva for 1 hour.
9:00 am	Fluoride for 1 min then rinsed for 30 seconds using DI water. Then immersed in 50:50 saliva for 1 hour.
10:00 am	Rinsed in DI water then placed in acid solution for 4 hours.
2:00 pm	Rinsed for 30 seconds using DI water then immersed in 50:50 saliva for 1 hour.
3:00 pm	30 seconds DI water rinse. Fluoride for 1 min then rinsed for 30 seconds using DI water. Then immersed in 50:50 saliva for 1 hour.
4:00 pm	30 seconds DI water rinse. Fluoride for 1 min then rinsed for 30 seconds using DI water. Then immersed in 50:50 saliva for 1 hour.

TABLE III

Summary of the mean and standard error for each outcome by group

	Control Demin (n = 8)	Group A (μ-CT n = 4, TMR n = 8)	Group B (μ-CT n = 5, TMR n = 8)	Group C (μ-CT n = 5, TMR n = 9)
μ-CT percent mineral recovery (%)		69 (14)	74 (8)	64 (11)
TMR IML (% volume mineral × μm)	1572 (104)	1012 (150)	1180 (138)	1157 (87)
TMR lesion depth (μm)	52 (3)	52 (3)	43 (4)	42 (3)

TABLE IV

The μ-CT sound ($\mu m^3 \times 10^5$) statistical data for each group

Group	N	Mean	SD	SE	Min	Max	95% CI for Mean	
Control Demin	4	1.64	0.89	0.44	0.55	2.42	0.23	3.05
Group A	4	2.90	1.03	0.51	1.72	4.1	1.27	4.54
Group B	5	1.42	0.64	0.29	0.78	2.36	0.62	2.22
Group C	5	1.04	0.42	0.19	0.44	1.46	0.51	1.56

TABLE V

The μ-CT demineralization ($\mu m^3 \times 10^5$) statistical data for each

Group	N	Mean	SD	SE	Min	Max	95% CI for Mean	
Control Demin	4	8.32	6.28	3.14	3.03	17.26	-1.67	18.31
Group A	5	9.67	2.4	1.07	6.44	12.69	6.68	12.65
Group B	5	6.47	5.42	2.42	2.80	15.9	-0.26	1.32
Group C	5	4.34	1.61	0.72	2.98	6.97	2.35	6.34

TABLE VI

The μ-CT remineralization ($\mu m^3 \times 10^5$) statistical data for each

Group	N	Mean	SD	SE	Min	Max	95% CI for Mean	
Group A	5	4.67	1.47	0.66	2.43	5.97	2.84	6.49
Group B	5	2.32	0.91	0.41	1.08	3.32	1.18	3.45
Group C	5	2.28	0.88	0.39	1.32	3.54	1.197	3.37

TABLE VII

The μ-CT percent mineral recovery (%) statistical data for each group

Group	N	Mean	SD	SE	Min	Max	95% CI for Mean	
Group A	4	69	28	14	27	90	24	114
Group B	5	74	18	8	57	96	52	95
Group C	5	64	24	11	37	91	34	94

TABLE VIII

TMR IML (%volume mineral × μm) statistical data for each group

Group	N	Mean	SD	SE	Min	Max	95% CI for Mean	
Control Demin	8	1572	293	104	1245	2114	1326	1817
Group A	8	1012	425	150	455	1861	657	1368
Group B	8	1180	390	138	498	1622	855	1506
Group C	9	1157	262	87	681	1463	956	1358

TABLE IX

TMR lesion depth (μm) statistical data for each group

Group	N	Mean	SD	SE	Min	Max	95% CI for Mean	
Control Demin	8	52	9	3	40	69	44	59
Group A	8	52	8	3	42	69	45	59
Group B	8	43	12	4	20	58	33	53
Group C	9	42	8	3	29	49	36	48

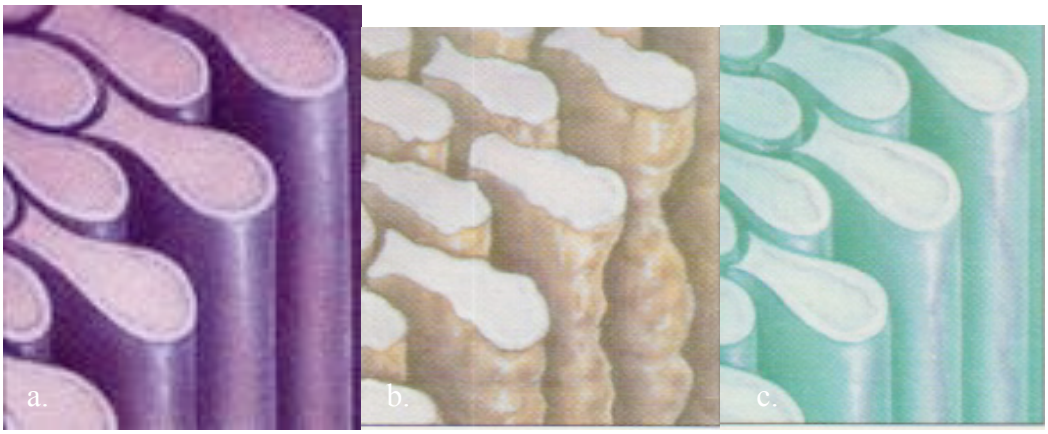

FIGURE 1. Schematic of enamel rod structure in sound, demineralized, and remineralized enamel: a) sound enamel; b) demineralized enamel, and c) remineralized enamel. (Copyright 2000 Martin S. Spiller, DMD)

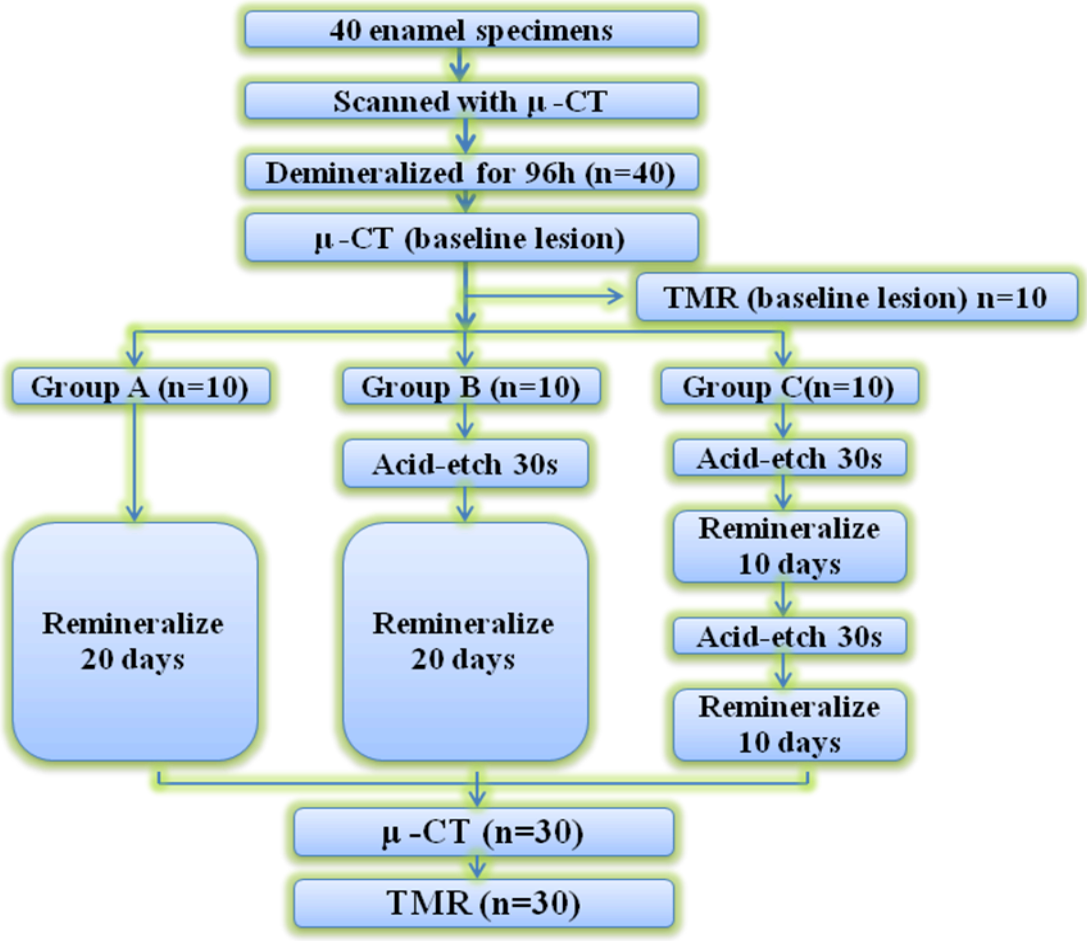

FIGURE 2. Diagram demonstrating the flow of procedures performed in this *in-vitro* study.

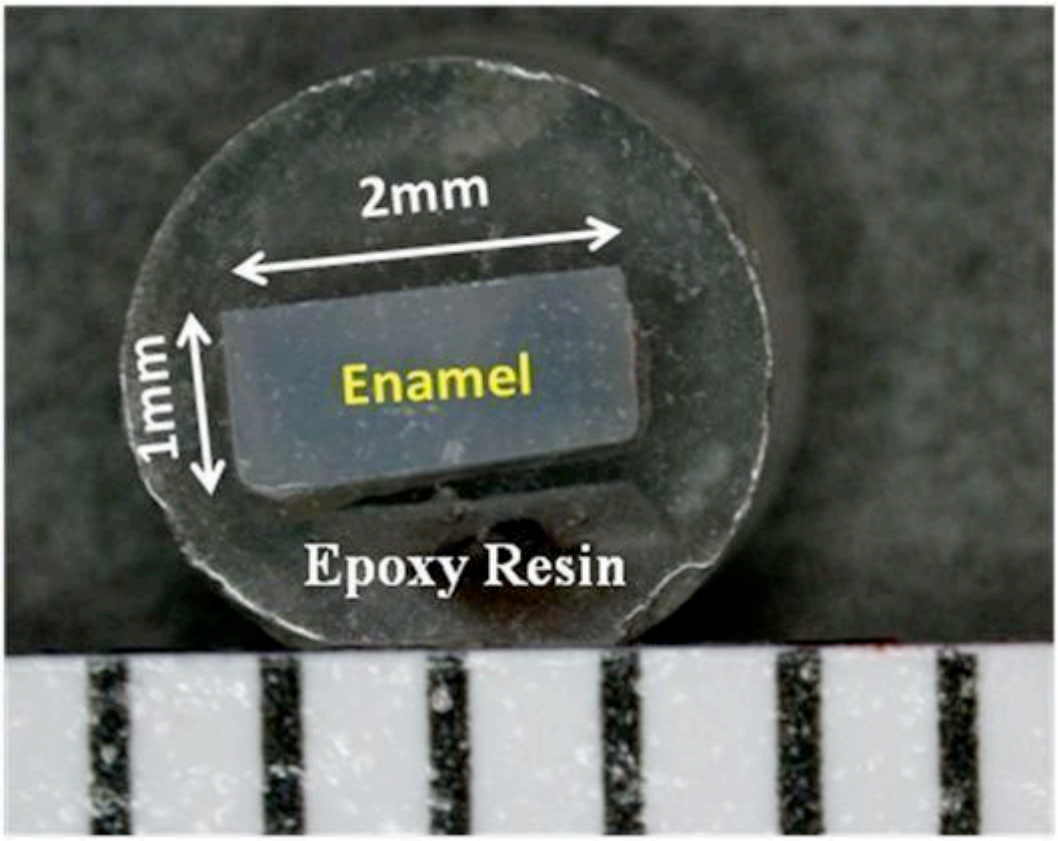

FIGURE 3. Enamel specimen embedded in epoxy resin.

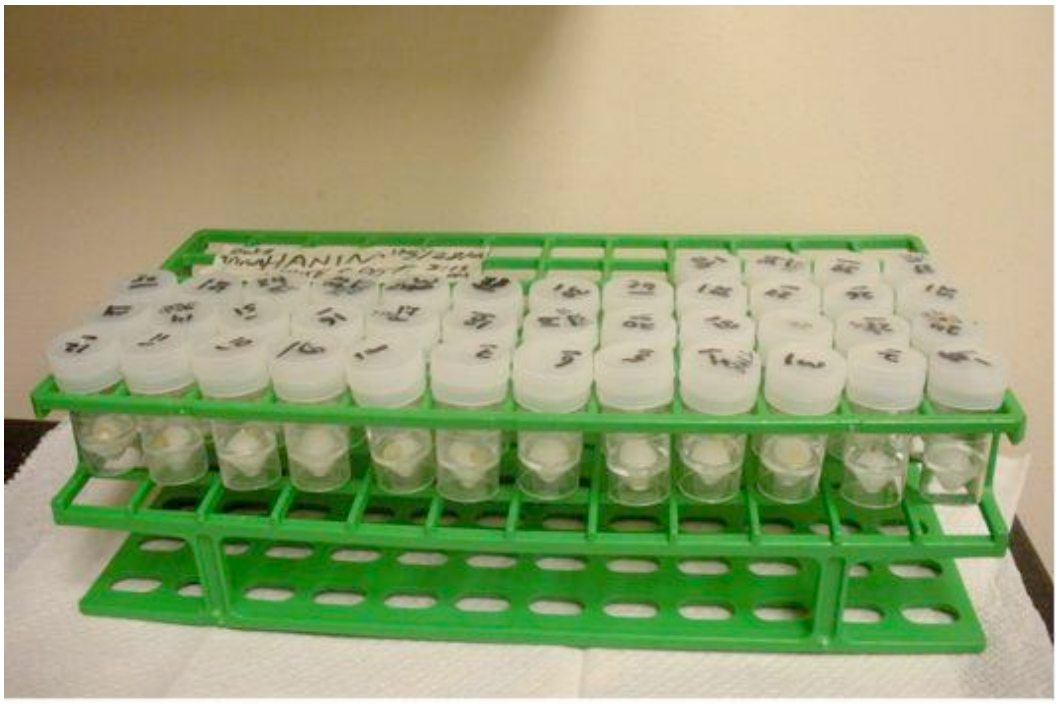

FIGURE 4. Specimens stored in separately marked vials with damp cotton pellets to preserve humidity.

FIGURE 5. Holder with sample position marked for CT scanning of samples.

FIGURE 6. SKYSCAN 1172 high resolution μ-CT.

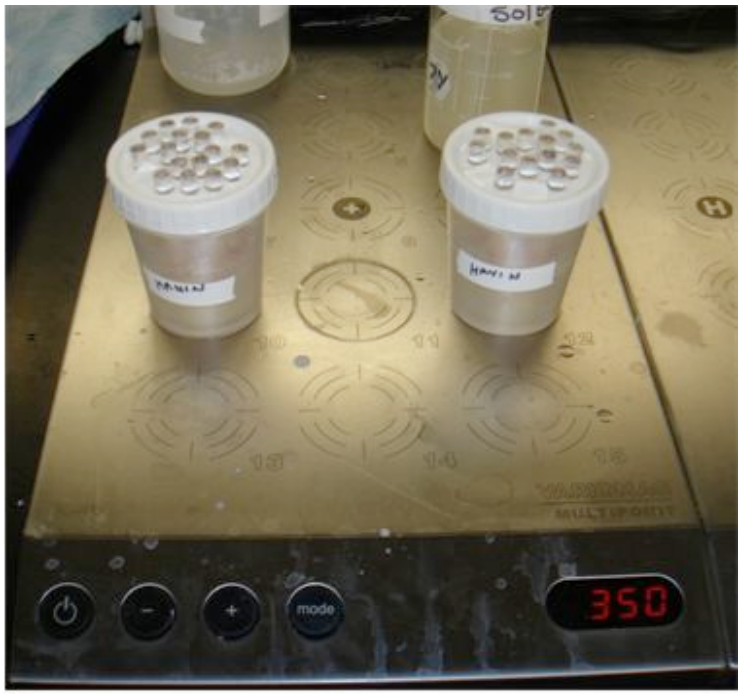

FIGURE 7. Enamel specimens mounted on plastic rods and submerged in 50/50 saliva in the remineralization/demineralization pH cycling model.

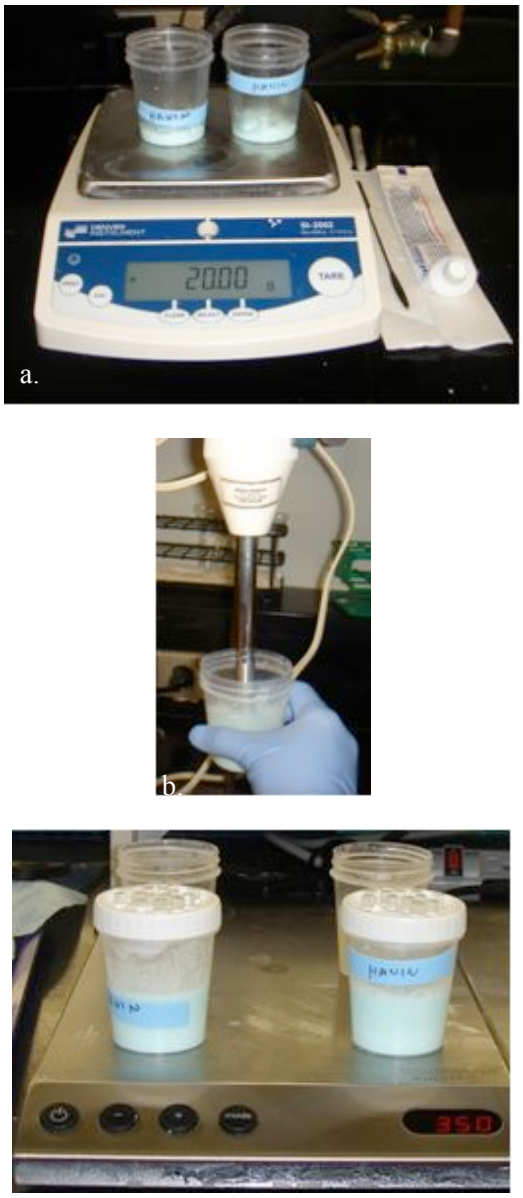

FIGURE 8. Sequence of procedures for the fluoride treatment phase of remineralization: a) weighing the fluoride toothpaste required for the mix; b) mixing of 50/50 saliva and fluoride toothpaste; c) specimens in fluoride mixture for 1 min.

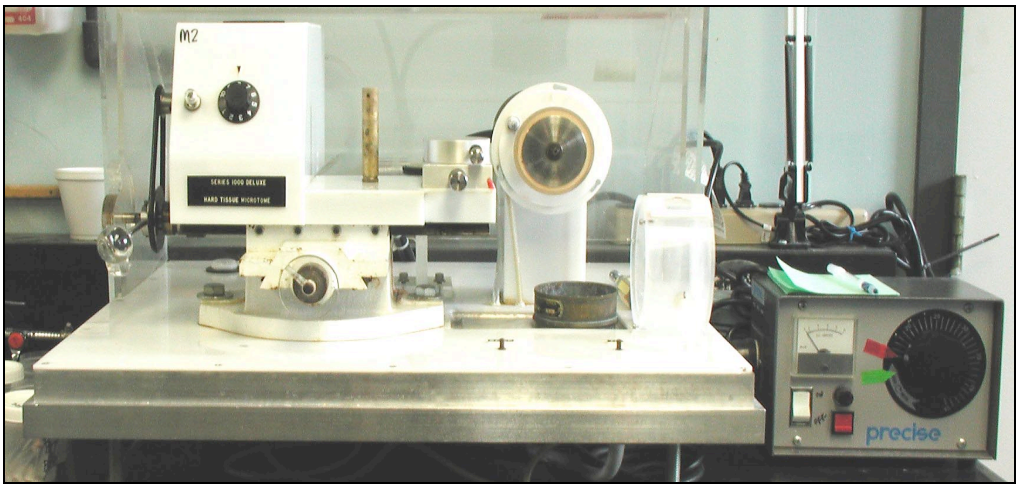

FIGURE 9. Series 1000 Deluxe Microtome used for obtaining thin sections for TMR analysis.

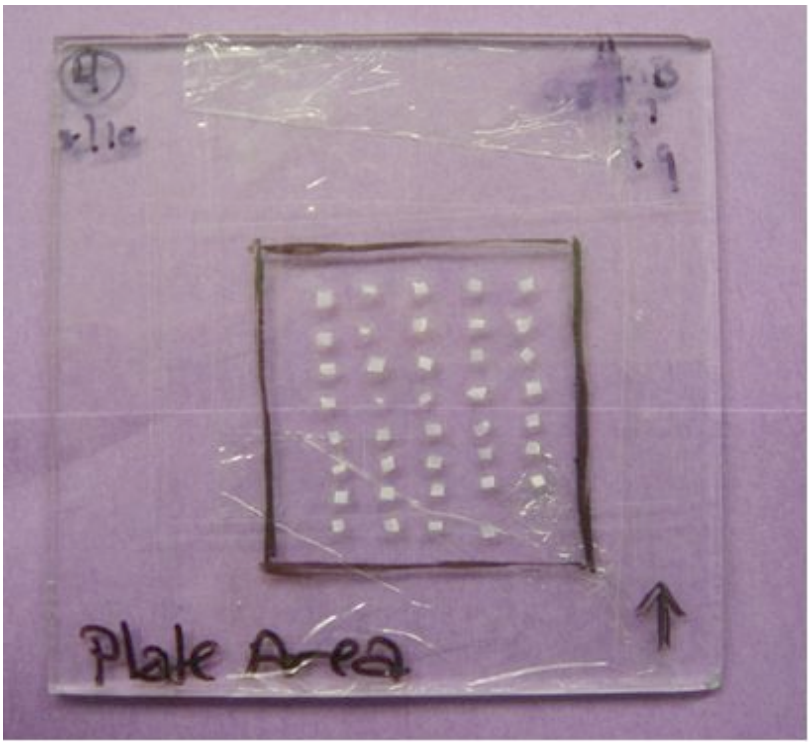

FIGURE 10. Enamel specimen 100-µm sections mounted on a glass plate for TMR analysis.

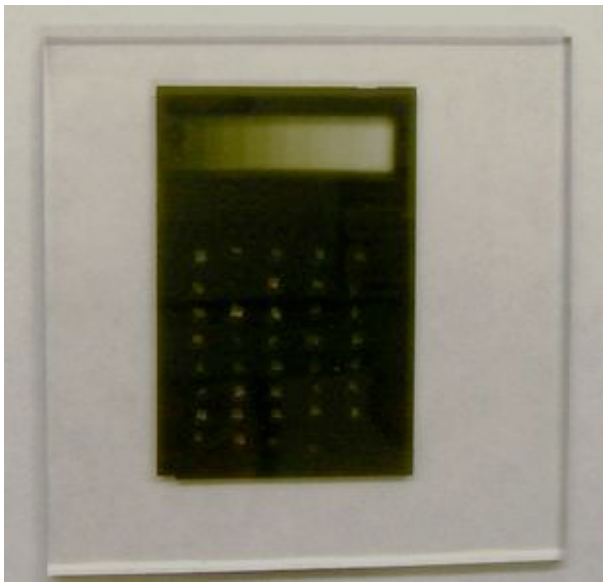

FIGURE 11. Radiographic plate after x-ray exposure and developing ready for TMR analysis.

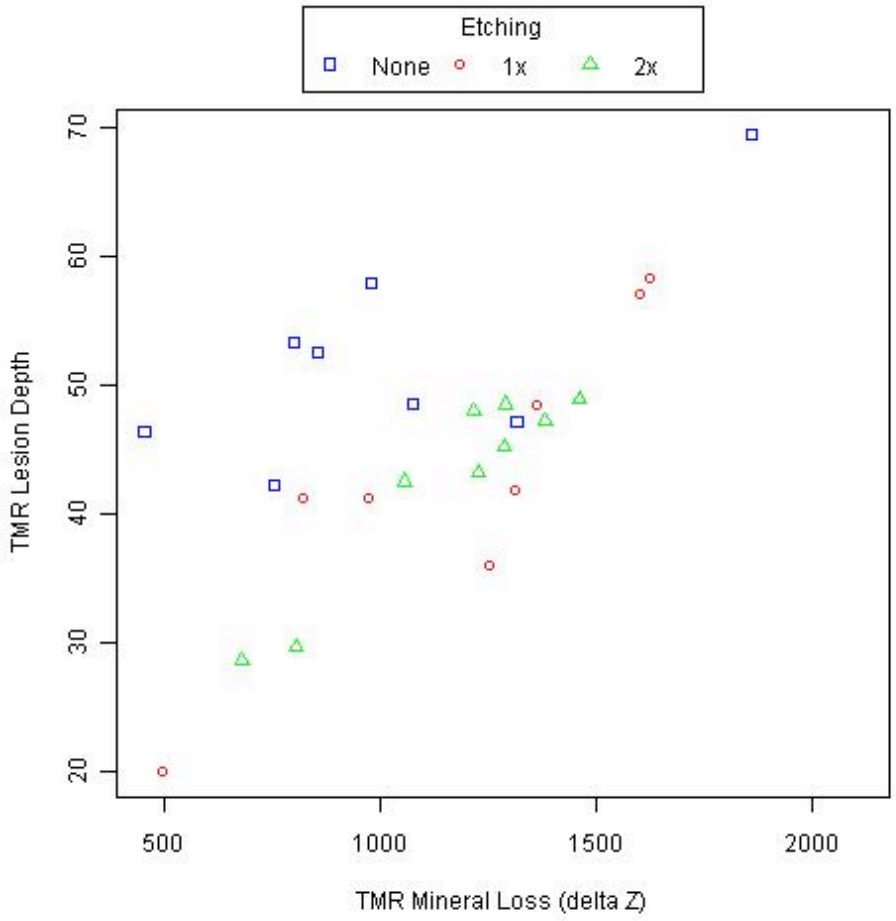

FIGURE 12. Graph demonstrating the correlation between TMR lesion depth and TMR mineral loss.

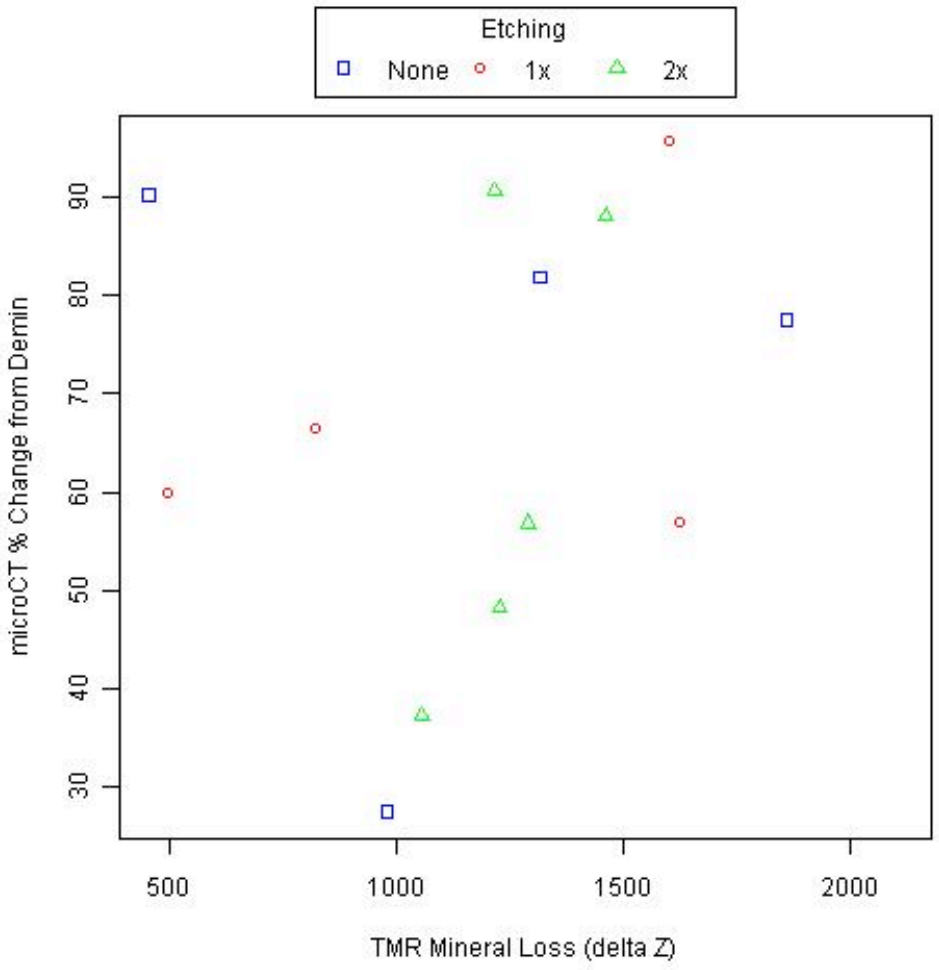

FIGURE 13. Graph demonstrating the lack of correlation between μ-CT percent mineral recovery and TMR mineral loss.

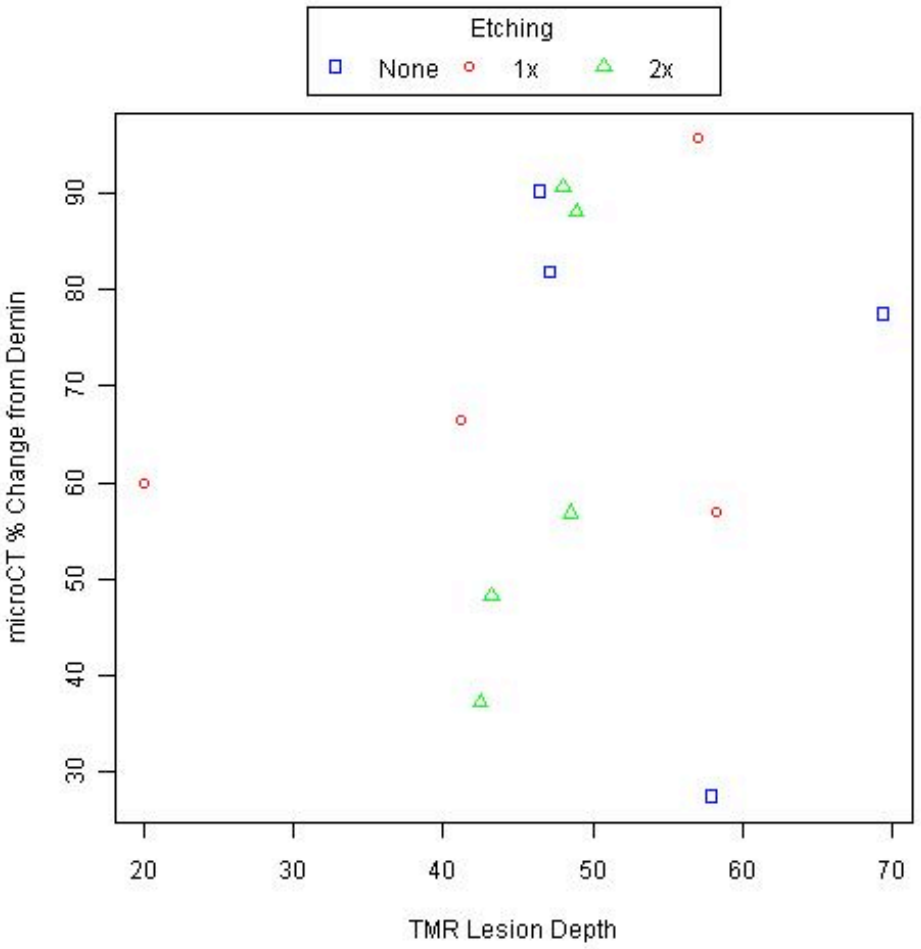

FIGURE 14. Graph demonstrating the lack of correlation between μ-CT percent mineral recovery and TMR lesion depth.

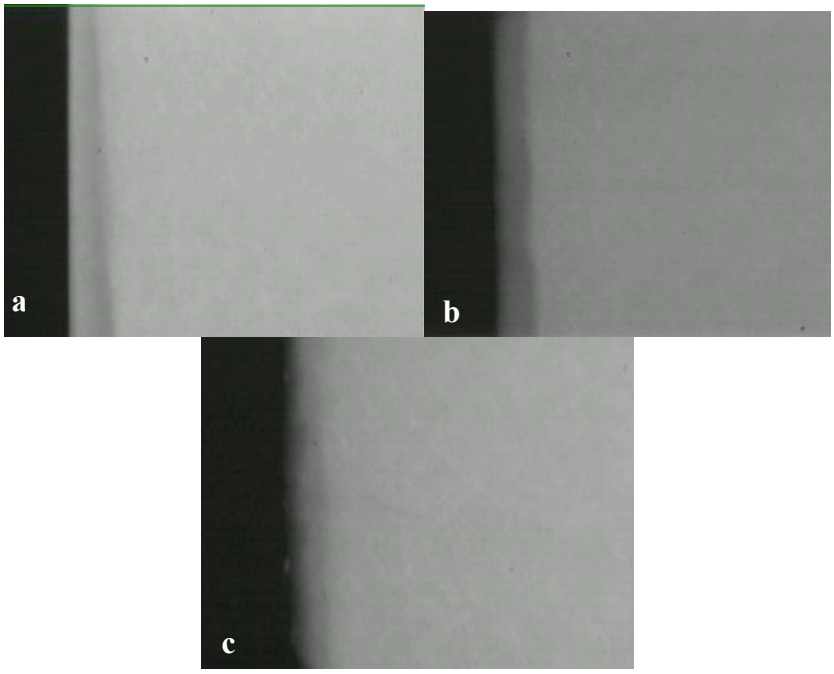

FIGURE 15. TMR images showing the lesion in the three remineralization groups: a) Group A lesion showing the continuous smooth surface layer, and b) Group B lesion showing the reduction of surface layer where acid-etching has affected it, and c) Group C lesion showing the significant reduction or loss of surface layer.

DISCUSSION

The most mineralized layer of incipient caries lesions is the surface layer.[3-5] One of the suggested reasons for the incomplete mineralization of the body of the lesion is the preferential deposition of minerals on the surface layer, which leads to a decrease in its porosity and prevents more minerals from reaching the deeper areas of the incipient enamel caries lesion. Acid etching of the surface layer was previously considered an option to create mineral passage pores, which would increase surface porosity enhancing the remineralization process. This enhancement was thought to be achieved by allowing easier access for the minerals to the body of the lesion without being hindered by the increasingly mineralized surface layer.[4, 7, 8, 42-44]

This study was formulated to investigate the remineralization potential of incipient enamel lesions in non-etched lesions, once-etched lesions, and lesions that were etched twice during the remineralization process. Both μ-CT and TMR were used to determine the mineral content and lesion depth of the enamel specimens. Additionally, the study aimed at investigating the ability of μ-CT to detect changes in mineral content in incipient caries lesions by comparing its resultant data with TMR data, the gold standard test for studies dealing with the mineralization of dental tissues.

In this study, a number of specimens were excluded from the final TMR analysis due to fractures that occurred during the final processing steps, which involved sectioning with the hard-tissue microtome and mounting on the radiographic plates for TMR analysis. These specimens included two specimens from each of the demineralization-only group (Control Demin); the remineralized, non-etched group (Group A); the

remineralized once-etched group (Group B), and one specimen from the remineralized, twice-etched group (Group C). The remaining specimens were included in the final analysis, in which each group was composed of eight specimens, except for Group C with nine specimens (Table III).

In the non-etched group, a highly remineralized surface layer was observed under the microscope as TMR analysis was being performed (Figure 15). This is supported by previous findings that relate surface layer formation to the fluoride-enhanced mineral precipitation from both the mineralization solution and the body of the lesion.[18, 35, 36] Fluoride has been demonstrated to draw free mineral ions from the lesion body towards the surface layer.[24, 29] This process allowed redistribution of the minerals throughout the lesion layers.[4]

Regardless of the treatment administered to the specimens, a substantial lesion remained at the end of the study, which indicated the absence of full remineralization. This finding was in accordance with the results of Al-Khateeb's study (2000).[4]

Although the results did not show a significant difference in TMR lesion depth among the tested groups, Groups B and C had the shallowest lesions (43 μm and 42 μm, respectively) compared with Group A and the Control Demin group (Table IX). This observation would lead us to question the ability of the acid-etching treatment to better reduce the size of incipient caries lesions. This is supported by previous studies that investigated the capacity of acidic media to facilitate remineralization of incipient caries lesions.[4, 6, 38]

The absence of a TMR lesion depth significantly different among Group A, Group B, Group C, and the Control Demin group suggests the limited, indicative capacity

of lesion depth to convey the extent of remineralization in incipient caries lesions. This would support the increased importance of the actual mineral amount in incipient lesions as opposed to the results obtained for lesion depth.

The TMR IML results show that the highest IML was in the Control Demin group, which indicates its specimens had the highest degree of demineralization compared with the remineralization groups, a finding that was significantly different from the other groups ($p < 0.05$). This would indicate that the remineralization procedures in the three experimental groups resulted in an increase in the mineral content of the lesions being tested, which is supported by the literature.[4-6, 21, 36, 79] The study results showed no significant difference among the remineralized groups, which would suggest the possible lack of an effect for acid etching on the remineralization process.

Although the remineralized groups had no TMR IML significant differences among them, groups B and C had the lowest degrees of remineralization compared with Group A, which received no acid-etching treatment. Group C specimens had even a low degree of remineralization compared with Group A specimens. This would suggest a reduction in the remineralization capacity of the acid-etched specimens, especially the twice-etched specimens, compared with the non-acid etched remineralization specimens. This suggestion could be in conflict with previous studies.[4, 6] Reviewing the TMR images of the specimens in each group would shed some light on the possible cause of such phenomena. In Figure 15(a), the non-acid etch treated lesion shows a thick, smooth and continuous surface layer followed by graduation into its deeper layers, which shows the infiltration of the minerals within the lesion during the remineralization process. In Figure 15(b), the acid etched-once, treated lesion shows a reduction in the surface layer,

which represents the acid effect on it. In Figure 15(c), the acid-etched, twice-treated lesion showed an increase in the surface-layer reduction along with the relative loss of it. These visual findings could reveal the reason behind the reduced remineralization in Group C. The possible explanation for this noticed reduction could be overwhelming damage to the surface layer of the incipient caries lesions under study. This would point out the possible important role that the surface layer plays in remineralization.[4,39] The use of a weaker acid or a reduction in the acid-etching time could be advisable to increase remineralization without destroying the surface layer.

During μ-CT analysis, a number of the specimens have been excluded from the final μ-CT study due to the inability to reproduce viable data from them to be used for lesion analysis. This can be attributed to the initially small size of the lesions that made it somewhat difficult to detect on reconstructed μ-CT images of these specimens, which might suggest this difficulty as a limitation of the use of μ-CT. Each group had five specimens excluded except for the Control Demin Group, which had six specimens excluded from its final analysis. The numbers of specimens in each group are included in Table III.

The μ-CT analysis data were used to calculate the mineral volume of 91-0 wt% in the selected area of the specimen. A higher resultant volume indicated a higher demineralization degree and mineral loss in that area. This could be related to surface microhardness test (SMH) data, in which the greater the resultant value, the greater the demineralization. This phenomenon is attributed to the effect created when the surface demineralizes and softens, causing the indentation implemented by the test to become longer.

The μ-CT analysis data showed that, generally, the highest mineral volume of 91-0 wt% was found in the demineralized specimens compared with both sound and remineralized specimens (Tables IV, V, and VI). This indicates the higher degree of demineralization and mineral volume loss in the demineralized specimens, which also indicates a lower degree of demineralization in the remineralized specimens. The lower degree of demineralization indicates some level of mineral recovery in the remineralized specimens. This finding agrees with the literature, which has confirmed that subjecting enamel specimens to fluoride-assisted remineralization solutions increases the mineral volume within the lesions being tested.[4, 6, 33, 36, 37]

To compare the mineral gain in the lesions among the groups by using μ-CT, we used percent mineral recovery (%) in 91-0 wt% as a measurement, which was calculated using the formula 100 × (remineralization mineral volume - demineralization mineral volume) / (sound mineral volume - demineralization mineral volume). This percentage would indicate the relative mineral gain in each group.

Although the study results revealed the absence of a significant difference among the three remineralized groups, the highest percent mineral recovery was found in Group B (Table VII). This would lead us to suspect a greater mineral gain in Group B lesions, which received an acid-etch treatment once, compared with that of Group A lesions, which received no acid-etch treatment. These speculations would agree with previous literature.[4, 6]

The results of the present study showed that the remineralization of Group C specimens was adversely affected, producing the lowest percent mineral recovery (64 %), in comparison with the other remineralized groups (Table III). This would lead us to

speculate that the high strength of the acid used for acid etching might have demineralized the surface layer to an overwhelming extent, instead of producing viable pores for mineral passage to the lesion body. The use of a less destructive acid, such as polyacrylic acid, or a lower concentration phosphoric acid, lower than 35 percent, might have produced a superior increase in remineralization without destroying the surface layer. Also, reduction of the acid-etching time from 30 seconds to a reduced treatment time might be a proposed solution to reduce the exposure time of the surface layer to the acid-etching agent. This reduction would preserve the surface layer to a better extent, while still allowing the formation of mineral access pores to the body of the lesion. The reduction in remineralization potential caused by destruction of the surface layer indicated the immensely important presence of the surface layer in order to achieve optimum and sustained remineralization results. Although the highly mineralized surface layer can act as a barrier that hinders remineralization,[4] its complete removal also introduces a huge burden that could terminate the remineralization process, thus causing similar effects to dental erosion, still considered in the literature as the hardest lesions to repair. Remnants of the surface layer are essential for remineralization of the less mineralized body of the lesion by providing a nucleation site that attracts minerals to the remineralizing lesion, a process also confirmed in the literature.[39]

This study demonstrated a lack of correlation between μ-CT and TMR IML as well as TMR lesion depth (Figures 13 and 14). This result suggests the need for further investigations into the accuracy of μ-CT measurements of minute mineral changes compared with TMR. A deeper, demineralized lesion might have also been needed for the μ-CT to detect subtle differences in lesion mineral content that later would have

better correlation with TMR IML and TMR lesion depth of enamel samples. This re-establishes the previously documented fact that TMR remains the gold standard for mineral studies of dental tissue experiments.[50, 51]

The correlation between TMR IML and TMR lesion depth was demonstrated by the study results as shown in Figure 12. This correlation would agree with previous findings establishing TMR as the gold standard with reproducible and well-correlated results, regardless of the size of the lesion under study.[50, 51] After reviewing the results, the null hypothesis was accepted, which proposed that there is no enhancement of mineral uptake in caries lesions by the application of an additional acid-etching treatment to the surface layer during the remineralization process.

In this study the *in-vitro* chemical model was used to produce subsurface lesions imitating incipient caries lesions formed in the oral cavity. This model was developed in previous studies to study the caries-preventive effects of different products. On the other hand, the microbial model was developed to better study the anti-microbial effects of different agents on caries development as well as the preventive effects of anti-caries treatments. Although *in-vitro* chemical models produce lesions in less time than other *in-vitro* and *in-vivo* models, the chemical model s have an inherent disadvantage of producing subsurface lesions likely to be more fragile and porous than most natural incipient lesions.[18,19] Therefore, the results of this study are related to this type of lesions. Different models might result in a different remineralization effect of the methods under study.

The present study's results indicate the great importance of preserving the integrity of the surface layer of incipient caries lesions in order to achieve optimum

remineralization results. Also, they suggest that additional measures might be needed to enhance the remineralization following the initial acid etching of enamel lesions. These might include the use of a higher-concentration fluoride treatment as well as a longer remineralization period. Given that the remineralization process is a process over time, the resultant remineralized specimens might have produced more variable remineralization degrees after a longer remineralization period, which would have demonstrated the remineralization potential of each group to a better extent.

SUMMARY AND CONCLUSIONS

This study was conducted to investigate the ability of an additional acid etch during the remineralization process to boost the remineralization results of incipient caries lesions. Further, we evaluated the ability of μ-CT to measure the mineral content changes in incipient caries lesions compared with TMR before and after the specimens were subjected to remineralization procedures.

In this study, three groups were compared with each other and with demineralized enamel specimens. The first group was subjected to fluoride-assisted remineralization, while the second group received an acid-etch treatment prior to the remineralization. The third group received an additional acid-etch treatment in the middle of the remineralization procedures.

Results from this study revealed no significant differences in μ-CT percent mineral recovery among the three groups, while TMR IML revealed a significant difference between the three groups collectively and the demineralized group. TMR lesion depth revealed no significant difference among all the groups, including the demineralized group. Additionally, no correlation was found between μ-CT percent mineral recovery and TMR IML and TMR lesion depth, although TMR lesion depth and TMR IML had a 0.66 correlation.

In spite of the statistically insignificantly different results, a decrease in mineral gain of the acid-etch-twice remineralization group was noticed in both μ-CT and TMR data analysis. This result was explained by the harsh effect of the acid-etching agent as seen in the TMR images (Figure 15).

From the presented results, we concluded that although the surface layer will hinder the process of remineralization in incipient caries lesions, the presence of the layer is nonetheless essential to achieving remineralization. Although the less porous surface layer interferes with the passage of minerals to the body of the lesion, the layer's remnants act as a protective barrier for the highly porous lesion's body, and provide remineralization nuclei, which attract minerals toward the body of lesion. Moreover, the mineral content of incipient caries lesions should be studied, and we recommend focusing on the ability of μ-CT to detect minute changes in mineral content. Regarding fluoride enhancement, additional measures could be required to improve the fluoride-induced remineralization process, because acid-etching might not be the ideal solution for every case, in view of the damage that could be done. Possible solutions include the use of lower-concentration acid etchants to induce remineralization without implementing the harsh demineralization effect. Finally, lesion depth should not be used as the sole indicator of the remineralization potential of various techniques and agents, because remineralization is better expressed in the amount of mineral gain within the lesion itself.

REFERENCES

1. Dean HT. Fluorine and dental caries. Ill Med J 1949;95(1):33-7.

2. Groeneveld A. Longitudinal study of prevalence of enamel lesions in a fluoridated and non-fluoridated area. Community Dent Oral Epidemiol 1985;13(3):159-63.

3. Collys K, Cleymaet R, Coomans D, Slop D. Acid-etched enamel surfaces after 24 h exposure to calcifying media in vitro and in vivo. J Dent 1991;19(4):230-5.

4. Al-Khateeb S, Exterkate R, Angmar-Mansson B, ten Cate JM. Effect of acid-etching on remineralization of enamel white spot lesions. Acta Odontol Scand 2000;58(1):31-6.

5. Yamazaki H, Litman A, Margolis HC. Effect of fluoride on artificial caries lesion progression and repair in human enamel: regulation of mineral deposition and dissolution under in vivo-like conditions. Arch Oral Biol 2007;52(2):110-20.

6. Flaitz CM, Hicks MJ. Role of the acid-etch technique in remineralization of caries-like lesions of enamel: a polarized light and scanning electron microscopic study. ASDC J Dent Child 1994;61(1):21-8.

7. Hicks MJ, Flaitz CM. Enamel caries formation and lesion progression with a fluoride dentifrice and a calcium-phosphate containing fluoride dentifrice: a polarized light microscopic study. ASDC J Dent Child 2000;67(1):21-8, 8.

8. Peariasamy K, Anderson P, Brook AH. A quantitative study of the effect of pumicing and etching on the remineralisation of enamel opacities. Int J Paediatr Dent 2001;11(3):193-200.

9. Mortimer KV. The relationship of deciduous enamel structure to dental disease. Caries Res 1970;4(3):206-23.

10. Wen SL. Human enamel structure studied by high resolution electron microscopy. Electron Microsc Rev 1989;2(1):1-16.

11. LeGeros RZ. Calcium phosphates in oral biology and medicine. Monogr Oral Sci 1991;15:1-201.

12. Ichijo T, Yamashita Y, Terashima T. Observations on the structural features and characteristics of biological apatite crystals. (Pt 1). Observation on the shape and arrangement of the cross sectioned enamel crystals. Dent Jpn (Tokyo) 1990;27(1):11-6.

13. Featherstone JD. Dental caries: a dynamic disease process. Aust Dent J 2008;53(3):286-91.

14. Arends J, Christoffersen J. The nature of early caries lesions in enamel. J Dent Res 1986;65(1):2-11.

15. Featherstone JD. The continuum of dental caries--evidence for a dynamic disease process. J Dent Res 2004;83 Spec No C:C39-42.

16. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994;8(2):263-71.

17. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50(4):353-80.

18. ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res 1982;16(3):201-10.

19. ten Cate JM, Mundorff-Shrestha SA. Working group report 1: Laboratory models for caries (in vitro and animal models). Adv Dent Res 1995;9(3):332-4.

20. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc 2000;131(7):887-99.

21. Silverstone LM. The significance of remineralization in caries prevention. J Can Dent Assoc 1984;50(2):157-67.

22. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res 1975;9(5):373-87.

23. Scott DB, Simmelink JW, Nygaard V. Structural aspects of dental caries. J Dent Res 1974;53(2):165-78.

24. Featherstone JD. Diffusion phenomena during artificial carious lesion formation. J Dent Res 1977;56 Special D:D48-52.

25. Featherstone JD, Rodgers BE. Effect of acetic, lactic and other organic acids on the formation of artificial carious lesions. Caries Res 1981;15(5):377-85.

26. Driessens FC, Heijligers HJ, Borggreven JM, Woltgens JH. Posteruptive maturation of tooth enamel studied with the electron microprobe. Caries Res 1985;19(5):390-5.

27. Silverstone LM. The surface zone in caries and in caries-like lesions produced in vitro. Br Dent J 1968;125(4):145-57.

28. ten Cate JM. Remineralization of caries lesions extending into dentin. J Dent Res 2001;80(5):1407-11.

29. ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med 1991;2(3):283-96.

30. Featherstone JD, Duncan JF, Cutress TW. A mechanism for dental caries based on chemical processes and diffusion phenomena during in-vitro caries simulation on human tooth enamel. Arch Oral Biol 1979;24(2):101-12.

31. Koch G. Prevalence of enamel mineralisation disturbances in an area with 1-1.2 ppm F in drinking water. Review and summary of a report published in Sweden in 1981. Eur J Paediatr Dent 2003;4(3):127-8.

32. Parfitt GJ. Fluorine in the diet as a factor in the prevention of dental caries. Postgrad Med J 1951;27(309):349-55.

33. Marinho VC, Higgins JP, Logan S, Sheiham A. Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2003(4):CD002782.

34. ten Cate JM. Remineralization of deep enamel dentine caries lesions. Aust Dent J 2008;53(3):281-5.

35. ten Cate JM, Jongebloed WL, Arends J. Remineralization of artificial enamel lesions in vitro. (Pt 4). Influence of fluorides and diphosphonates on short- and long-term reimineralization. Caries Res 1981;15(1):60-9.

36. Schemehorn BR, Orban JC, Wood GD, Fischer GM, Winston AE. Remineralization by fluoride enhanced with calcium and phosphate ingredients. J Clin Dent 1999;10(1 Spec No):13-6.

37. Lynch RJ, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. Int Dent J 2004;54(5 Suppl 1):304-9.

38. Yamazaki H, Margolis HC. Enhanced enamel remineralization under acidic conditions in vitro. J Dent Res 2008;87(6):569-74.

39. Larsen MJ, Fejerskov O. Chemical and structural challenges in remineralization of dental enamel lesions. Scand J Dent Res 1989;97(4):285-96.

40. Dalzell DP, Howes RI, Hubler PM. Microabrasion: effect of time, number of applications, and pressure on enamel loss. Pediatr Dent 1995;17(3):207-11.

41. Croll TP, Killian CM, Miller AS. Effect of enamel microabrasion compound on human gingiva: report of a case. Quintessence Int 1990;21(12):959-63.

42. Hicks MJ, Silverstone LM, Flaitz CM. A scanning electron microscopic and polarized light microscopic study of acid-etching of caries-like lesions in human tooth enamel treated with sodium fluoride in vitro. Arch Oral Biol 1984;29(10):765-72.

43. Belser U, Sporri S, Muhlemann HR. Uptake and retention of fluoride by intact and etched enamel. Helv Odontol Acta 1975;19(2):69-71.

44. DePaola PF, Aasenden R, Brudevold F. The use of topically applied acidulated phosphate-fluoride preceded by mild etching of the enamel: a one-year clinical trial. Arch Oral Biol 1971;16(10):1155-63.

45. van Dorp CS, Exterkate RA, ten Cate JM. Mineral loss during etching of enamel lesions. Caries Res 1990;24(1):6-10.

46. Kato K, Nakagaki H, Sakakibara Y, Kameyama Y, Weatherell JA, Robinson C. Distribution of fluoride in the enamel of rat incisors examined by an abrasive microsampling technique. Arch Oral Biol 1988;33(9):653-6.

47. Ten Bosch JJ, Angmar-Mansson B. A review of quantitative methods for studies of mineral content of intra-oral caries lesions. J Dent Res 1991;70(1):2-14.

48. Willmott NS, Wong FS, Davis GR. An X-ray microtomography study on the mineral concentration of carious dentine removed during cavity preparation in deciduous molars. Caries Res 2007;41(2):129-34.

49. Davis GR, Wong FS. X-ray microtomography of bones and teeth. Physiol Meas 1996;17(3):121-46.

50. Meyer-Lueckel H, Paris S. Progression of artificial enamel caries lesions after infiltration with experimental light curing resins. Caries Res 2008;42(2):117-24.

51. Damen JJ, Exterkate RA, ten Cate JM. Reproducibility of TMR for the determination of longitudinal mineral changes in dental hard tissues. Adv Dent Res 1997;11(4):415-9.

52. Angmar B CD, Glas J-E. . Studies on the ultrastructure of dental enamel. (Pt 4). The mineralisation of normal human enamel. J Ultrastruct Res 1963;8:12-23.

53. Mellberg JR, Castrovince LA, Rotsides ID. In vivo remineralization by a monofluorophosphate dentifrice as determined with a thin-section sandwich method. J Dent Res 1986;65(8):1078-83.

54. Exterkate RA, Damen JJ, ten Cate JM. A single-section model for enamel de- and remineralization studies. (Pt 1). The effects of different Ca/P ratios in remineralization solutions. J Dent Res 1993;72(12):1599-603.

55. Thomas RZ, Ruben JL, de Vries J, ten Bosch JJ, Huysmans MC. Transversal wavelength-independent microradiography, a method for monitoring caries lesions over time, validated with transversal microradiography. Caries Res 2006;40(4):281-91.

56. Lagerweij MD, de Josselin de Jong E, ten Cate JM. The video camera compared with the densitometer as a scanning device for microradiography. Caries Res 1994;28(5):353-62.

57. Huysmans MC, Thomas RZ. [Research methods in dentistry. (Pt 6). In vivo and in vitro methods for studying caries lesion progression]. Ned Tijdschr Tandheelkd 2004;111(12):471-6.

58. Kim I, Paik KS, Lee SP. Quantitative evaluation of the accuracy of micro-computed tomography in tooth measurement. Clin Anat 2007;20(1):27-34.

59. Wong FS, Anderson P, Fan H, Davis GR. X-ray microtomographic study of mineral concentration distribution in deciduous enamel. Arch Oral Biol 2004;49(11):937-44.

60. Hahn SK, Kim JW, Lee SH, Kim CC, Hahn SH, Jang KT. Microcomputed tomographic assessment of chemomechanical caries removal. Caries Res 2004;38(1):75-8.

61. Stauber M, Muller R. Micro-computed tomography: a method for the non-destructive evaluation of the three-dimensional structure of biological specimens. Methods Mol Biol 2008;455:273-92.

62. Postnov AA, Vinogradov AV, Van Dyck D, Saveliev SV, De Clerck NM. Quantitative analysis of bone mineral content by x-ray microtomography. Physiol Meas 2003;24(1):165-78.

63. Huang TT, Jones AS, He LH, Darendeliler MA, Swain MV. Characterization of enamel white spot lesions using X-ray microtomography. J Dent 2007;35(9):737-43.

64. Schwass DR, Swain MV, Purton DG, Leichter JW. A system of calibrating microtomography for use in caries research. Caries Res 2009;43(4):314-21.

65. Kazakia GJ, Burghardt AJ, Cheung S, Majumdar S. Assessment of bone tissue mineralization by conventional x-ray microcomputed tomography: comparison with synchrotron radiation microcomputed tomography and ash measurements. Med Phys 2008;35(7):3170-9.

66. Kato A, Ohno N. Construction of three-dimensional tooth model by micro-computed tomography and application for data sharing. Clin Oral Investig 2009; 13(1):43-6. Epub 2008 Apr 3.

67. Wong FS, Elliott JC, Anderson P, Davis GR. Mineral concentration gradients in rat femoral diaphyses measured by x-ray microtomography. Calcif Tissue Int 1995;56(1):62-70.

68. Chappard D, Basle MF, Legrand E, Audran M. Trabecular bone microarchitecture: a review. Morphologie 2008;92(299):162-70.

69. Dedrick DK, Goldstein SA, Brandt KD, O'Connor BL, Goulet RW, Albrecht M. A longitudinal study of subchondral plate and trabecular bone in cruciate-

deficient dogs with osteoarthritis followed up for 54 months. Arthritis Rheum 1993;36(10):1460-7.

70. Wong FS, Willmott NS, Davis GR. Dentinal carious lesion in three dimensions. Int J Paediatr Dent 2006;16(6):419-23.

71. Dowker SE, Elliott JC, Davis GR, Wassif HS. Longitudinal study of the three-dimensional development of subsurface enamel lesions during in vitro demineralization. Caries Res 2003;37(4):237-45.

72. Rhodes JS, Ford TR, Lynch JA, Liepins PJ, Curtis RV. Microcomputed tomography: a new tool for experimental endodontology. Int Endod J 1999;32(3):165-70.

73. Harris DA, Jones AS, Darendeliler MA. Physical properties of root cementum. (Pt 8). Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: a microcomputed tomography scan study. Am J Orthod Dentofacial Orthop 2006;130(5):639-47.

74. Foo M, Jones A, Darendeliler MA. Physical properties of root cementum. (Pt 9). Effect of systemic fluoride intake on root resorption in rats. Am J Orthod Dentofacial Orthop 2007;131(1):34-43.

75. Clementino-Luedemann TN, Kunzelmann KH. Mineral concentration of natural human teeth by a commercial micro-CT. Dent Mater J 2006;25(1):113-9.

76. Zou W, Gao J, Jones AS, Hunter N, Swain MV. Characterization of a novel calibration method for mineral density determination of dentine by x-ray micro-tomography. Analyst 2009;134(1):72-9.

77. Schweizer S, Hattendorf B, Schneider P, et al. Preparation and characterization of calibration standards for bone density determination by microcomputed tomography. Analyst 2007;132(10):1040-5.

78. Bonse U, Busch F, Gunnewig O, et al. 3D computed x-ray tomography of human cancellous bone at 8 microns spatial and 10(-4) energy resolution. Bone Miner 1994;25(1):25-38.

79. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80(12):2066-70.

ABSTRACT

THE EFFECT OF ACID ETCHING ON REMINERALIZATION OF INCIPIENT CARIES LESIONS: A MICRO-CT STUDY

by

Hanin Essam Yeslam

Indiana University School of Dentistry
Indianapolis, Indiana

Etching of enamel caries lesions has been demonstrated to enhance remineralization. However, this effect reaches a plateau after a period of time. This study aimed at investigating the effectiveness of additional acid etching on remineralization.

Forty 1 mm × 2 mm human enamel blocks with chemically induced artificial incipient lesions were used. Ten specimens were randomly selected at the end of demineralization for transverse microradiography (TMR) analysis. The remaining specimens were then divided into three groups (n = 10). Group A was remineralized by a pH cycling system with 1100 ppm sodium fluoride for 20 days. In group B, the specimens were etched with 35-percent phosphoric acid for 30 s and then remineralized. Group C was remineralized by same procedure as group B plus and given an additional acid etch after 10 days of remineralization. Mineral density was measured by x-ray

microtomography (μ-CT). The volumetric mineral content [VM ($\mu m^3 \times 10^5$)] was determined between 91 and 0-wt%. The μ-CT % mineral recovery (%) was calculated using the formula 100×(remineralize VM - demineralization VM) / (sound VM - demineralization VM). One-hundred-μm sections of demineralized and remineralized specimens were used to assess the mineral loss (IML: vol%×μm) and lesion depth (μm) using TMR.

The three groups showed no significant difference in mineral change or mineral content for μ-CT or TMR lesion depth. The TMR IML showed a significant difference between the demineralized specimens and the three remineralized groups. The correlation between TMR IML and TMR lesion depth was 0.66 ($p < 0.0001$). The μ-CT percent mineral recovery from demineralization was correlated with neither TMR IML nor TMR lesion depth. When evaluated with μ-CT, the twice-acid-etched group presented lower mineral gain values than the group etched only once with acid. Also, the twice-etched group presented lower mineral gain and greater TMR IML compared with the non-acid etch group. TMR images revealed reduction of surface layer in the acid-etched groups, especially in the twice-etched group, in which significant reduction or loss of surface layer occurred.

Based on these results, we conclude that additional acid etching with 35-percent phosphoric acid does not enhance remineralization compared with a single application of acid etching. We believe that the viable existence of the surface layer is essential for remineralization of the lesion. Further investigations into the accuracy of μ-CT to detect minute mineral changes in incipient caries lesions are probably needed.

CURRICULUM VITAE

Hanin Essam Yeslam

July 2004	BDS King AbdulAziz University School of Dentistry Jeddah, Saudi Arabia
July 2005	Internship year King AbdulAziz University School of Dentistry Jeddah, Saudi Arabia
August 2005	GP Dentist NorthWest Armed Forces Hospital Tabuk, Saudi Arabia
June 2007	Teacher's Assistant King AbdulAziz University School of Dentistry, Operative Dentistry Department Jeddah, Saudi Arabia
December 2009	MSD Graduate Operative Program, Indiana University School of Dentistry Indianapolis, IN

Professional Organizations

Saudi Dental Association
American Dental Association (ADA)
Academy of Operative Dentistry