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Academic Journals

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Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

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Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998;

1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

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Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Journal of Dentistry and Oral Hygiene

Table of Content: Volume 5   Number 7   July   2013

ARTICLES

Research Articles

Full Length Research Paper

Dental erosion in patients with gastro esophageal reflux disease (GERD)

Golsa Alavi[1], Ali A. Alavi[1], Mehdi Saberfiroozi[2], Amir H. Sarbazi[1] and Mehran Motamedi[1]*

[1]Department of Operative Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Gastroenterology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

The aim of this study was to investigate the effects of gastro esophageal reflux disease (GERD) on dental erosion. The study group comprised 140 patients with the average age of 30 to 50 years old. All subjects completed a detailed questionnaire regarding dental and medical situations. Dental examination was performed by two examiners agreement method, and endoscopy was done by a gastroenterologist. Patients were divided into three groups of healthy, suspected of GERD, and with GERD. Data were collected and analyzed by Chi-Square test. The prevalence of dental erosion in GERD patients (22.6%) was found to be higher than suspected (5.3%) and healthy (7%) subjects. This study has showed that GERD patients are at increased risk of developing dental erosion compared to healthy subjects.

Key words: Dental erosion, reflux, heart burn.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a situation in which the esophagus is injured or swelled due to the return of stomach acid fluids. Esophagus is located behind the heart, so the term "heart burn" is usually used to describe the condition (Shi et al., 1995). Most of the patients complained of heart burn at least once a month, and a few percent have more incidents even up to once a day. More than 36% of the United States adult population and its prevalence increases after the age of 40, and not only the adults, but also the children and infants, might suffer from the disease (Wienbeck and Bamert, 1989; Sonnenberg and El-Serag, 1999). Stomach produces hydro chloric (HCl) acid in order to digest the foods and the epithelial cells lining its internal surface are protected against this acid by extensive mucosal secretions. Esophageal epithelial cells lack this defensive mechanism and are prone to injury from the acid. In normal conditions, a muscular ring (sphincter) in the lowest end of the esophagus, located at the junction of the esophagus and the stomach, prevents acid from returning upwards. During swallowing, this sphincter is relax, allowing the food enter the stomach. But after swallowing, this sphincter closes the way to prevent the food from returning to the esophagus. In GERD, the sphincter is relaxed even during swallowing, so, stomach fluids containing strong acids, injure esophageal lining cells. Etiology of the problem relates to factors which help relaxing the described sphincter. It includes life style, tobacco or alcohol abuse, obesity, and also some drugs including calcium (Ca) channel blockers, theophilin, nitrates and antihistamines. In addition, some foods like fried foods, chocolates, onion and garlic, caffeinated drinks, acidic food, tomato, sour foods and mint can attribute to the disease. Habits such as high volumes of food intake especially just before sleeping, and diseases such as hiatal hernia, diabetes and pregnancy are also risk factors (Barham et al., 1995).

*Corresponding author. E-mail: mmotamedi@sums.ac.ir.

Symptoms of GERD include heart burn, acid reflux, difficulty in swallowing (dysphasia) which often worsens after having a meal and lasts for 2 h, although sports or heavy activities do not start or worsen the situation, but sleeping and bending the body downwards will.

GERD has some oral and dental complications and the most common is increased dental erosion (Wienbeck and Bamert, 1989; Sonnenberg and El-Serag, 1999). Dental erosion is irreversible loss of enamel due to the chemical reactions not related to the bacterial actions (Sullivan and Kramer, 1983). Tooth enamel is the hardest substance in the whole body, but it can be dissolved chemically in a solution. Acids causing dental erosion may have originated from the inside of the body (like reflux or vomiting) or from the outside (like acidic drinks or fruits). Unlike attrition which occurs on specific locations, erosion affects all surfaces of the teeth and can lead to discoloration, tooth sensitivity, shortened or rounded teeth, fractured teeth, and even tooth loss.

Dentists often ask the patient's medical history and GERD is in concern because of its dental complications. With the cooperation of the dentist and the gastro-enterologist, in addition to identifying the disease, teeth could be maintained for a longer period of time by an appropriate treatment plan (Lazarchik and Filler, 1997).

MATERIALS AND METHODS

This study was performed based on:

1. All the patients who were referred to the endoscopy center of the Namazi Hospital, and were visited by two dentists; questionnaire (Appendix 1) was filled by dentists.
2. Then, questionnaire (Appendix 2) was filled by the patient while being observed by an endoscopy center's specialist.
3. Endoscopy was performed for all the patients by a specialist.
4. Questionnaires (Appendices 1 and 2) were evaluated by two dentists and analyzed by a statistics specialist.

Current study evaluated the possible relation between the dental erosion and GERD. 140 patients from department of endoscopy, Shiraz Namazi Hospital were evaluated. Two separate questionnaires were designed for each subject. The first included personal information, oral hygiene, and dental evaluation. Dental evaluation was performed concerning caries, extracted teeth, restored teeth, replaced teeth and gingival bleeding done by two examiners with examiner agreement method. Dental radiographies were not taken and inter-proximal caries were excluded from the study. The second questionnaire included personal information and clinical data regarding reflux. The gastroenterologist after endoscopy, divided the patients into three groups – healthy (71 subjects), suspected to reflux (38 subjects), and having reflux (31 subjects).

After coding the questionnaires, data were transferred to the SPSS (statistical package for social science) software for analyze by chi-square test. Also, statistical analyses including cross tabulation test were done to compare the variants and discover the correlations. The statistical significance was defined as P<0.05.

RESULTS

In the first group, 5 patients (7%) had dental erosion whereas the number of subjects having dental erosion in the second and third group was 2 (5.3%) and 7 (22.6%), respectively (Table1). Among the 3 groups, according to chi-square test, the P-value was less than 0.05 in group 3, indicating the statistical relationship between the reflux and dental erosion, meaning that if a patient has GERD, the risk of dental erosion will increase (Table 2 and Figure 1).

DISCUSSION

Acid have destructive effects on the tooth structure and can be originated from intrinsic or extrinsic sources. If the acid contacts the tooth for a longer period than the tolerance of the enamel, enamel will be demineralized and the process is called dental erosion. Dentists discovered long ago that children suffering from reflux and recurrent vomits have this lesion and this has been observed in adults too. In a study by Ersin et al. (2006) on GERD effect on dental erosion, caries and saliva on 38 children, it was found that in the patients having GERD, incidence of erosion, salivary yeast and MS colonization was apparently higher than the normal population, in agreement with current study on adult subjects. Dental Practice Education Research Unit (2003) in a recommended oral care program has stated that patients with dental erosion of intrinsic origin often suffer from GERD and are under treatment, suggesting these lesions should be treated. David (2006) believes that GERD can occur at any age and might cause erosion of the enamel because of hydrochloric acid contact with the enamel when the stomach contents return into the mouth. Family Gentle Dental Care (2008) has also agreed to our findings. American Dental Association (2008) named some factors which start or worsen dental erosion: drinks, GERD and maintaining aspirin and vitamin C tablets in the mouth for long periods of time. According to Moazzez (2005), acid reflux in GERD patients can lead to erosion, and gum chewing which increases swallowing cycles, can help empty esophagus and thus reduce acidic postprandial esophageal reflux. In spite of most dentists who believe GERD as the major etiologic factor in formation of dental erosion, results from studies are difficult to compare because of scoring system, samples, and examiners. Males have more dental erosions than the females and maxillary anterior teeth are the mostly affected teeth (Academy of General Dentistry [AGD impact], 2007).

Imfeld (1996) has suggested the prevention of dental erosion by reducing acidic foods consumption, increasing saliva, fluoride administration, having foods with buffering activity, not to use materials and instruments which may erode the teeth and finally, appropriate restorations. Chu et al. (2002) have also evaluated treatment modalities for erosion with regard to the clinical and material considera-tion in order to achieve healthy, esthetic and functional teeth. Yip et al. (2002) have showed that erosion in the

Table 1. Reflux* erosion cross tabulation.

Reflux		Erosion		Total
		0	1	
Group 1	Count	66	5	71
	% within reflux	93.0	7.0	100.0
	% within erosion	52.4	35.7	50.7
	% of total	47.1	3.6	50.7
	Residual	2.1	-2.1	-
Group 2	Count	36	2	38
	% within reflux	94.7	5.3	100.0
	% within erosion	28.6	14.3	27.1
	% of total	25.7	1.4	27.1
	Residual	1.8	-1.8	-
Group 3	Count	24	7	31
	% within reflux	77.4	22.6	100.0
	% within erosion	19.0	50.0	22.1
	% of total	17.1	5.0	22.1
	Residual	-3.9	3.9	-
Total	Count	126	14	140
	% within reflux	90.0	10.0	100.0
	% within erosion	100.0	100.0	100.0
	% of total	90.0	10.0	100.0

Group 1: healthy; Group 2: suspected to reflux; Group 3: having reflux.

Table 2. Chi-square tests.

Variable	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	7.002[a]	1	0.008	-	-
Continuity correction[b]	5.322	1	0.021	-	-
Likelihood ratio	5.929	1	0.015	-	-
Fisher's exact test	-	-	-	0.015	0.015
Linear-by-linear association	6.952	1	0.008	-	-
No. of valid cases[b]	140	-	-	-	-

[a]1 cell (25.0%) that have expected count less than 5. The minimum expected count is 3.10; [b]Computed only for a 2×2 table.

United States is less common than the United Kingdom and Europe, and is a major factor in tooth destruction in young population. They discovered acidic foods and reflux from the stomach as primary etiologic factors for emphasizing on prevention rather than treatment.

Conclusion

This study was performed to evaluate oral manifestations, especially dental erosion in relation to GERD in Shiraz Namazi Hospital to which many patients are referred from
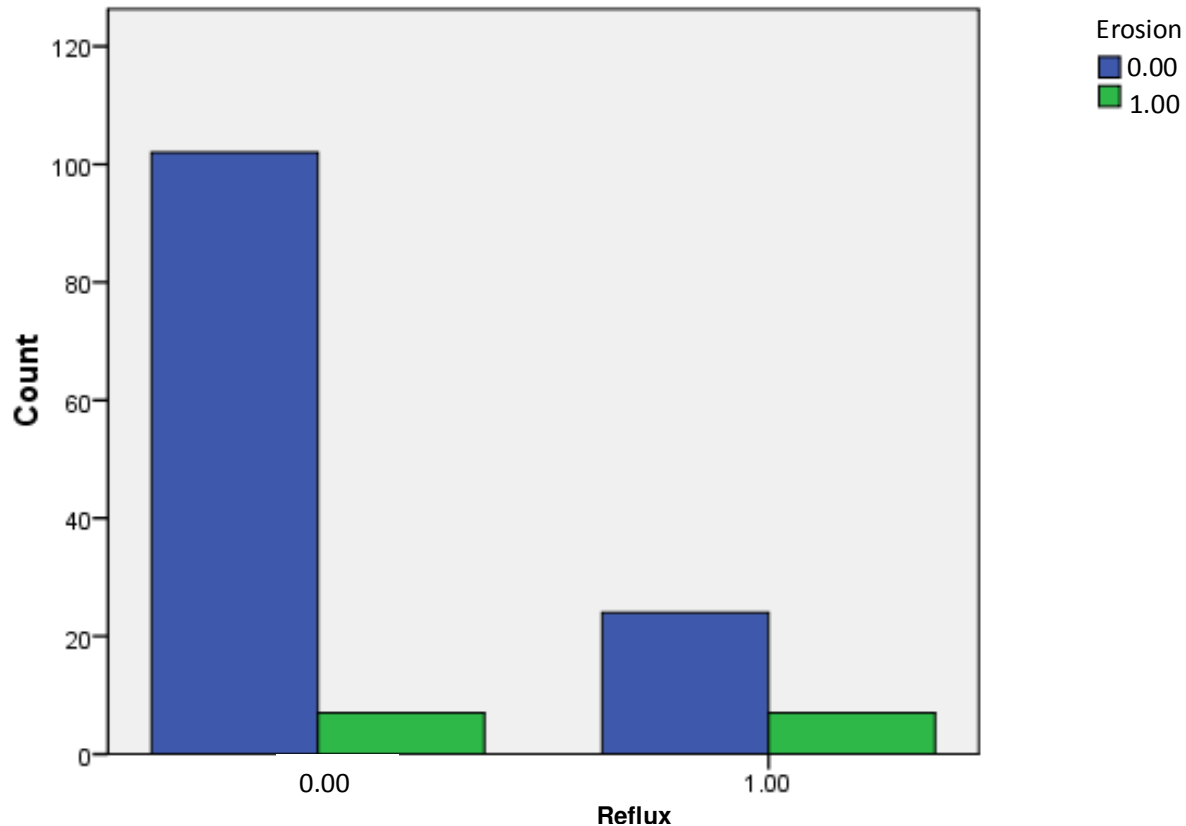
Figure 1. Chi-square bar chart.

local and neighboring cities. In this study, 22.6% of subjects with GERD also had dental erosion, and with p value of less than 0.05, a statistical relationship was found between GERD and dental erosion.

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Appendix 1

First Name: Last Name: Date of birth:

Age: occupation: Degree of education: Place of birth:

1. How many times you brush your teeth during a day?

One time □   two times □   sometimes □   I don't brush my teeth □

2. How many times do you use mouthwash during a week?

One time □   two times □   sometimes □   I don't use mouthwash □

3. How many times do you go to a dentist during a year?

One time □   two times □   more than two times □   never □

4. Have you ever been under radiotherapy?

Yes □   No □

5. Which drugs do you consume?

Caries:	NO □	Yes □
Erosion:	NO □	Yes □
Filling:	NO □	Yes □
Extraction:	NO □	Yes □
Partial:		
Denture crown:	NO □	Yes □
Bleeding while brushing:	NO □	Yes □
Smoker:	NO □	Yes □
Drink coke:	NO □	Yes □
Systemic disease:	NO □	Yes □

Appendix 2

Name and last name…………………   Cod: ………………………………

1. Sex:		1- Male □	2- Female □		
2. Age: …………….. date of birth					
3. Marital status	1- unmarried □	2- married □			
4. Address:					
5. Do you suffer regurgitation (Heart burn)?		1- Yes □	2- No □		
5/1. If yes, for how long? Months……………… years………………					
5/2. If yes, how many times? 1. Once and more daily 2. 3 to 6 times a week 3. Once or twice a week 4. Once or more a month 5. Less than once a month					
5/3. If yes, fill the questionnaire:					

Complaint	Negative	Positive		
		Mild	Moderate	Severe
Heart burn				
Acid reflux				
Food reflux				
Heart burn without cardiac involvement				
Problem in swallowing				
Odynophagial				
Diurnal				
Vomiting				
Nocturnal apnea				
Chronic sore throat				
Nocturnal coughing				
Nocturnal dyspnea				
Whizzing				

Mild: There is no need for treatment; Moderate: Treatment is required but daily activities are not affected; Severe: Permanent treatment is required, daily activities are inhibited.

Journal of Dentistry and Oral Hygiene

Full Length Research Paper

In vitro assessments of white-spot lesions treated with NaF plus tricalcium phosphate (TCP) toothpastes using microtomography (micro-CT)

Makoto Asaizumi[1]*, Robert L. Karlinsey[2], Allen C. Mackey[2], Tomoaki Kato[3] and Tetsuya Kuga[3]

[1]Dr. Makoto Asaizumi Orthodontic Practice, Ban Bldg. 3F. 1-8 Chiyodacho, Mobara, Chiba 297-0023, Japan.
[2]Indiana Nanotech, 351 West 10th Street, Suite 309, Indianapolis, IN 46202-4119, USA.
[3]Dr. Tetsuya Kuga Dental Practice, 924 Kamiichiba, Mutsuzawamachi, Chouseigun, Chiba 299-4403, Japan.

X-ray microtomography (micro-CT) was used to assess the densities of white-spot lesions (WSL) treated with either a fluoride-free paste, or 0.21 or 1.1% NaF toothpastes containing functionalized tricalcium phosphate (TCP). Bovine enamel specimens were ground, polished and demineralized to form WSL. Specimens (N=10) were treated with one of the following NaF silica-based toothpastes in a 10-day pH cycling model: (1) Tom's of Maine (0% NaF), (2) Clinpro® Tooth Crème (0.21% NaF plus TCP), and (3) Clinpro® 5000 (1.1% NaF plus TCP). Each day consisted of four 2 min treatments, one 4 h acid challenge (pH=5.0), and immersion in artificial saliva (pH=7.0) between these events. After cycling, specimens were analyzed using micro-CT. Statistical analysis (Student's t-test, Welch t-test or Tukey HSD test) was performed at the 95% confidence level. Compared to sound enamel, significantly ($p<0.05$) lower densities in WSL were only found at 24 µm from Clinpro Tooth Crème, and at 36 µm from Clinpro 5000. In contrast, the densities measured at 12, 24, 36, and 48 µm from the fluoride-free toothpaste were lower ($p<0.05$) than sound enamel. Micro-CT analyses revealed NaF toothpastes containing TCP led to increased WSL densities relative to the fluoride-free toothpaste.

Key words: Toothpaste, microtomography (micro-CT), density, remineralization.

INTRODUCTION

Caries decay in tooth enamel remains a worldwide problem, and international global summits are organized to bring research together from locations around the world to examine the evidence and plan strategic efforts to deal with changes in dental caries prevalence (Bagramian et al., 2009). Orthodontists have to select patients very carefully and provide detailed oral hygiene instructions. When combined with dietary advice by an auxillary at regular intervals throughout treatment, this remains the most cost-effective approach (Mitchell, 1992). However, these approaches may not be sufficient, so other strategies are often employed, such as at-home topical fluoride formulations. In the United States, dental practitioners may recommend professional-strength 1.1% sodium fluoride tooth pastes for high-risk patients instead of the typical range in fluoride toothpaste (e.g. 0.20 to 0.32% NaF) readily available over-the-counter (Nordström and Birkhed, 2009). For instance, it has been estimated that using professional-strength and over-the-counter fluoride toothpastes leads to an average clinical reduction in caries by approximately 70 and 22%, respectively (Tavss et al., 2003). While such therapies might be recommended for

various at-risk dental populations, from an orthodontic perspective, the inherent cleaning difficulties that are the direct result of the installation of dental appliances could lead to the recommendation of a high-fluoride treatment and/or multiple-step treatment regime to help fight against tooth decay. A relatively new and promising approach is the incorporation of a fluoride-compatible functionalized tricalcium phosphate (TCP) ingredient, which is a hybrid material that comprised of β–tricalcium phosphate and sodium lauryl sulfate, to NaF formulations (Karlinsey and Pfarrer, 2012). In particular, 3 M ESPE's Clinpro® Tooth Crème (0.21% NaF) and Clinpro® 5000 (1.1% NaF), which contain the TCP ingredient, are two of the newest professional-grade toothpastes commercially available. Inclusion of the func-tionalized TCP ingredient in NaF formulations has been shown to produce stronger, more acid-resistant mineral relative to fluoride alone in laboratory and clinical evalua-tions (Karlinsey et al., 2010a, b, c, 2009a, b, 2011a; Amaechi et al., 2012).

While transverse microradiography, cross-polarization microscopy and microhardness measurements are valuable in the assessment of remineralization/demineralization, another method of assessing efficacy may be through the use of X-ray microtomography (micro-CT). Micro-CT has been utilized in the observation of time-course changes in remineralization (Nakata et al., 2012) and demineralization (Watanabe et al., 2012); however, the use of micro-CT to assess changes in density of white-spot lesions (WSL) treated with NaF plus TCP systems has not yet been reported. As such, the purpose of this *in vitro* study was to use micro-CT to assess the densities of WSL treated with one of the following dentifrices: a fluoride-free, TCP-free control, or a 0.21 or 1.1% sodium fluoride toothpaste containing TCP. These densities were then compared to those measured for baseline sound and WSL enamel.

MATERIALS AND METHODS

Specimen preparation and WSL formation

Three millimeter enamel cores were drilled from the lingual and labial surfaces of bovine incisors using a bench top drill press (Power Glide, China) affixed with a hollow core drill bit. The cores were embedded into hollowed out acrylic rods using DuraBase resin (Reliance Dental Mfg. Co., Worth, IL, USA). Each specimen was ground by hand with 600 grit SiC sandpaper under water cooling for 30 s using a Leco Spectrum System 1000 Grinder/Polisher (St. Joseph, MI, USA) set to 300 rpm. Then, with the unit set to 200 rpm, each specimen was polished by hand for 1 min using 3 µm diamond compound in conjunction with microid extender solution (Leco). After rinsing with distilled (DI) water, Vickers surface micro hardness was performed with a 200 gf load and 15 s dwell time (Leco LM247AT micro hardness tester, St. Joseph, MI, USA) to confirm the presence of sound enamel in the polished specimen as indicated by a Vickers hardness number (VHN) of 300 VHN or greater. Acceptable sound specimens were then immersed in vials containing a 0.2 wt% 450 kDa polyacrylic acid (Sigma-Aldrich, St. Louis, MO, USA) and 0.1 M lactic acid (Sigma-Aldrich) solution saturated 50% with hydroxyapatite

(BioRad, Hercules, CA, USA) and adjusted to a pH of 5.0 (Karlinsey and Pfarrer, 2012). These vials were subsequently loaded into an incubator for 40 h at 37°C to establish 'white-spot' (non-cavitated) lesions, the characteristics and histology of which have been discussed in detail previously by White (1987). Briefly, the ensuing caries-like lesions produced by this solution have a dense surface mineral zone approximately 15 µm thickness, while the lesion depth extends to about 70 µm (White, 1987). Upon white-spot formation, acceptable baseline surface micro hardness ranging from 25 to 50 VHN (200 gf for 15 s made using a LecoLM247AT indenter) were included in the study.

Treatment groups and study protocol

Bovine enamel specimens (N=10 per group) were then grouped for treatment with one of the following silica-containing dentifrices: toothpaste A, Tom's of Maine fluoride-free toothpaste; toothpaste B, Clinpro® Tooth Crème (0.21% NaF plus TCP); toothpaste C, Clinpro® 5000 (1.1% NaF plus TCP); sound (N=10) and WSL (N=10) specimens were also produced for control purposes.

As outlined in Table 1, the three groups of enamel specimens were then cycled in a remin/demin pH cycling model lasting 10 days. This daily cycling model comprised immersion of inverted specimens in two 2 min treatment events performed an hour apart in the morning, followed by one four-hour polyacrylic acid-lactic acid challenge (15 mL, pH=5.0), and finally two more two-minute treatment events in the afternoon. Specimens were inverted and immersed in artificial saliva in between the daily treatments and acid challenge, as well as overnight (Cate, 1988). The treatments were diluted three-fold with DI water (5 g dentifrice: 10 ml DI water) to create the dentifrice slurry. The treatments and saliva events were magnetically agitated at 300 rpm, while the acid challenge was static. After each treatment and acid challenge, the specimens were rinsed with DI water prior to placement into artificial saliva. Fresh treatment slurries and acid solution were used daily, with the artificial saliva solution changed once daily during the acid challenge.

Micro-CT analysis

A commercial micro-CT (Toscaner-30000 µhd®, Toshiba, Japan) was used for density measurement at Advanced Composite Technology Center, Japan Aerospace Exploration Agency (JAXA) in Tokyo, Japan. The parameters used to collect micro-CT data, which were optimized based on the best image collected, are shown in Table 2. The 'Laks' (standard parameters) filter function was used to probe densities of specimens. An example view of the micro-CT apparatus and the mounted specimen fixed to the turn table is as shown in Figure 1. From this setup, an example 'field of view' (FOV) image was taken, as shown in Figure 2. The raw data from the 50 bovine enamel specimens (N=10 for each treatment groups, plus 10 baseline sound and 10 baseline WSL) were collected, the window level and width were adjusted, and then they were converted to bitmap files using Toscaner-30000V.17.46 operating software. CTAn® software version 1.12.0 (SkyScan, U.S.A.) was used to determine density based on the 256 gray scale collected from each enamel specimen. Data reconstruction did not include ring free correction (RFC). Five regions of interest (ROI) were randomly selected from one slice picture indicated at the position 50.0 ± 1.0 grayscale value (Figure 3). The grayscale baseline was set at 50.0 points ± 1.0 in ROI based on the brightness generated from the surface in one circular ROI from the top-view perspective as shown in Figure 3. Since the bovine enamel surfaces were not perfectly flat, a small ROI helped to reduce inclinations at the top surface. In doing so the possibilities of involving ring and/or beam-

Table 1. Outline of daily events and duration employed in the remin/demin pH cycling model.

Event	Duration
Treatment 1*	2 min
Saliva, pH=7.0	1 h
Treatment 2	2min
Saliva, pH=7.0	1 h
Acid challenge, pH=5.0	4 h
Saliva, pH=7.0	1 h
Treatment 3	2 min
Saliva, pH=7.0	1 h
Treatment 4	2 min
Saliva, pH=7.0	Overnight

On day one, specimens were pre-conditioned for 1 h in artificial saliva prior to the first treatment. Artificial saliva refreshed daily after the acid challenge. The treatment will consist of a three-fold dilution of test product to water (that is, 5 g paste, 10 ml distilled water). The carbopol-lactic acid system will be used to prepare the artificial lesions and will also be used throughout the 10-day cycling study.

Table 2. Micro-CT exposure parameters.

Parameter	Value
Scan. time (s)	355.8
Recon. time (s)	5.7
Tube voltage (kV)	90.000
Tube current (mA)	385.0000
number of views	800
Integration number	3
FPD gain (pF)	0.25
FPD integration time (ms)	124
FOV (mm)	6.987782
Slice thickness (mm)	0.0120
System name	TOSCANER-30000
Matrix size	1024
Scan mode	Half
Filter function	Laks
RFC	none
Image bias	0
Image slope	100
Data mode	Cone beam
Image direction	From the top
FDD	800.000
FCD	16.500
Window level	767
Window width	1230
Pixel size (mm)	0.0068240059
Magnification ratio	40.92858
Scale (mm)	0.6987782

hardening artifacts in the ROIs also were reduced (Figure 4), and careful consideration was used in selecting ROIs lacking these types of artifacts. Densities were then determined at each slice depth at baseline, $z1=12$ μm, $z2=24$ μm, $z3=36$ μm, $z4=48$ μm, $z5=60$ μm, $z6=72$ μm, and $z7=84$ μm. Only toothpaste B group (that is, Clinpro Tooth Crème) had specimens that produced X-rays out of range of the FOV: in this instance, only eight of the ten specimens provided data and were measured at 40 places with 8

slice depths per place for a total of 320 ROIs. Otherwise, all ten specimens from each of the other two toothpaste groups, along with the baseline sound and baseline WSL, were measured at 50 places with 8 slice depths per place for a total of 400 ROIs. To obtain absolute densities, the baseline density was then subtracted from the z1 through z7 densities for each group.

Statistics

All statistics were determined using the statistical package SAS-JMP (SAS Institute, USA). The mean densities at each depth (z1 through z7) for each of the dentifrice groups (A, B and C) along with the two control groups (sound and baseline WSL) were defined as independent variables. Comparisons between groups were made by comparing either the baseline sound enamel or the baseline WSL as the control group to the WSL treated with each of the three toothpastes as the experimental group. Each measurement was considered of equivalent variance so parametric testing (Student's t-test) of the mean densities between the control groups and experimental groups were performed, with $p<0.05$ considered significant. For multiple comparison testing, one-way analysis of variance (ANOVA) was used followed by Tukey highest significant difference (HSD) test to determine where the differences existed ($p<0.05$).

RESULTS

Density versus slice depth comparison between sound enamel and toothpaste-treated groups

Mean density values at each slice depth were compared between baseline sound enamel and lesions treated with either Clinpro Tooth Crème (Figure 5), Clinpro 5000 (Figure 6) or Tom's of Maine fluoride-free toothpaste (Figure 7). Significant differences were found between sound enamel and lesions treated with Clinpro Tooth Crème at z2 and z7 (Figure 5). At these slice depths, the mean density of lesions treated with Clinpro Tooth Crème was significantly lower ($p<0.05$) at z2 and significantly higher ($p<0.05$) at z7 compared to baseline sound enamel. With respect to comparisons between sound enamel and lesions treated with Clinpro 5000 (Figure 6), the mean density of lesions treated with Clinpro 5000 was significantly lower ($p<0.05$) than the baseline sound enamel at the z3 slice depth. Comparisons of density values versus slice depth between sound enamel and lesions treated with Tom's of Maine (Figure 7) revealed the density values were significantly lower ($p<0.05$) at z1, z2, z3 and z4 for lesions treated with Tom's of Maine.

Density versus slice depth comparison between WSL and toothpaste-treated groups

Mean density values at each slice depth were compared between baseline WSL and lesions treated with either Clinpro Tooth Crème (Figure 5), Clinpro 5000 (Figure 6) or Tom's of Maine fluoride-free toothpaste (Figure 7). Significant differences were found between baseline WSL
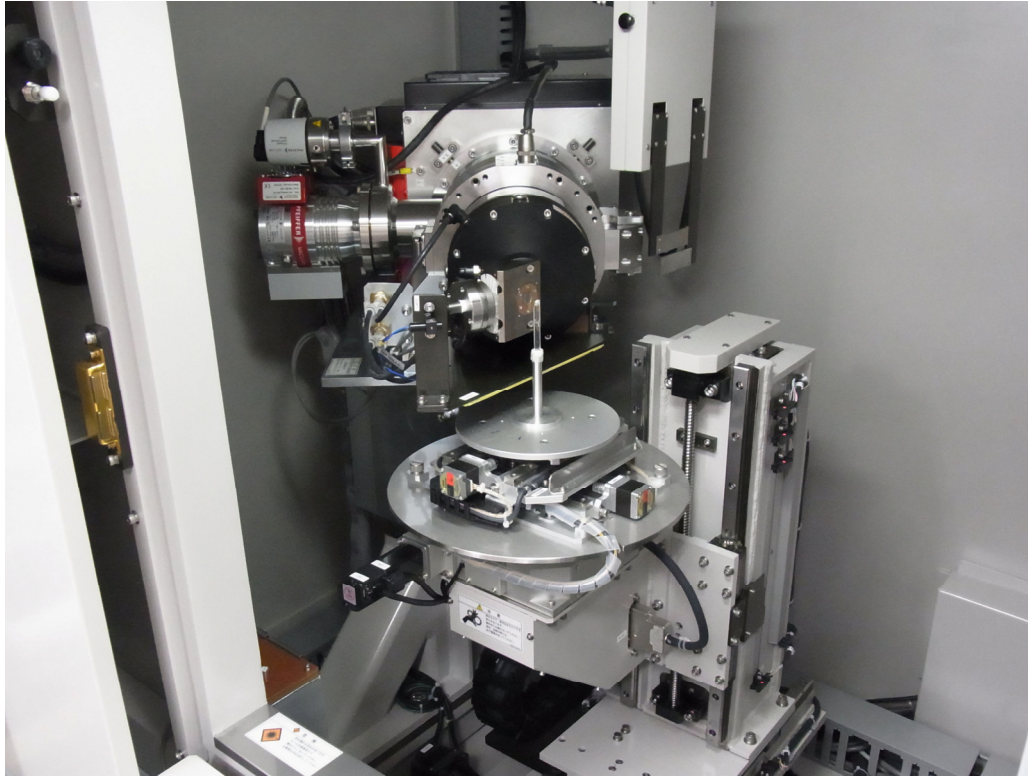
Figure 1. Representative view of an enamel specimen positioned on the turntable in the micro-CT apparatus.
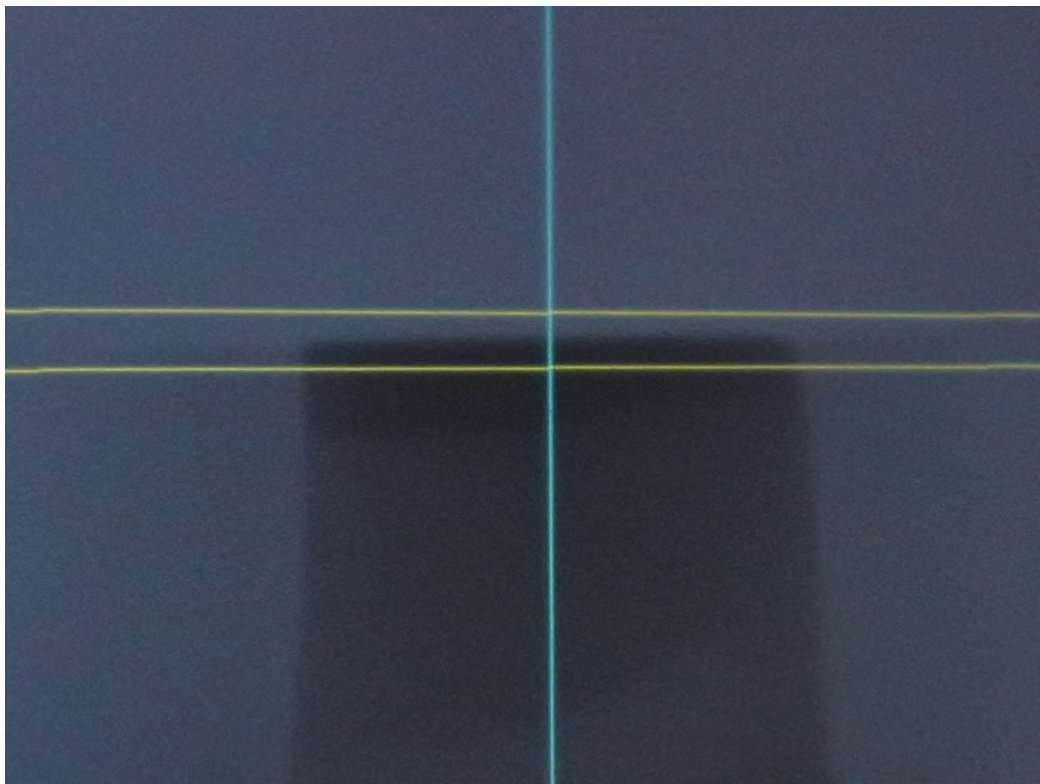

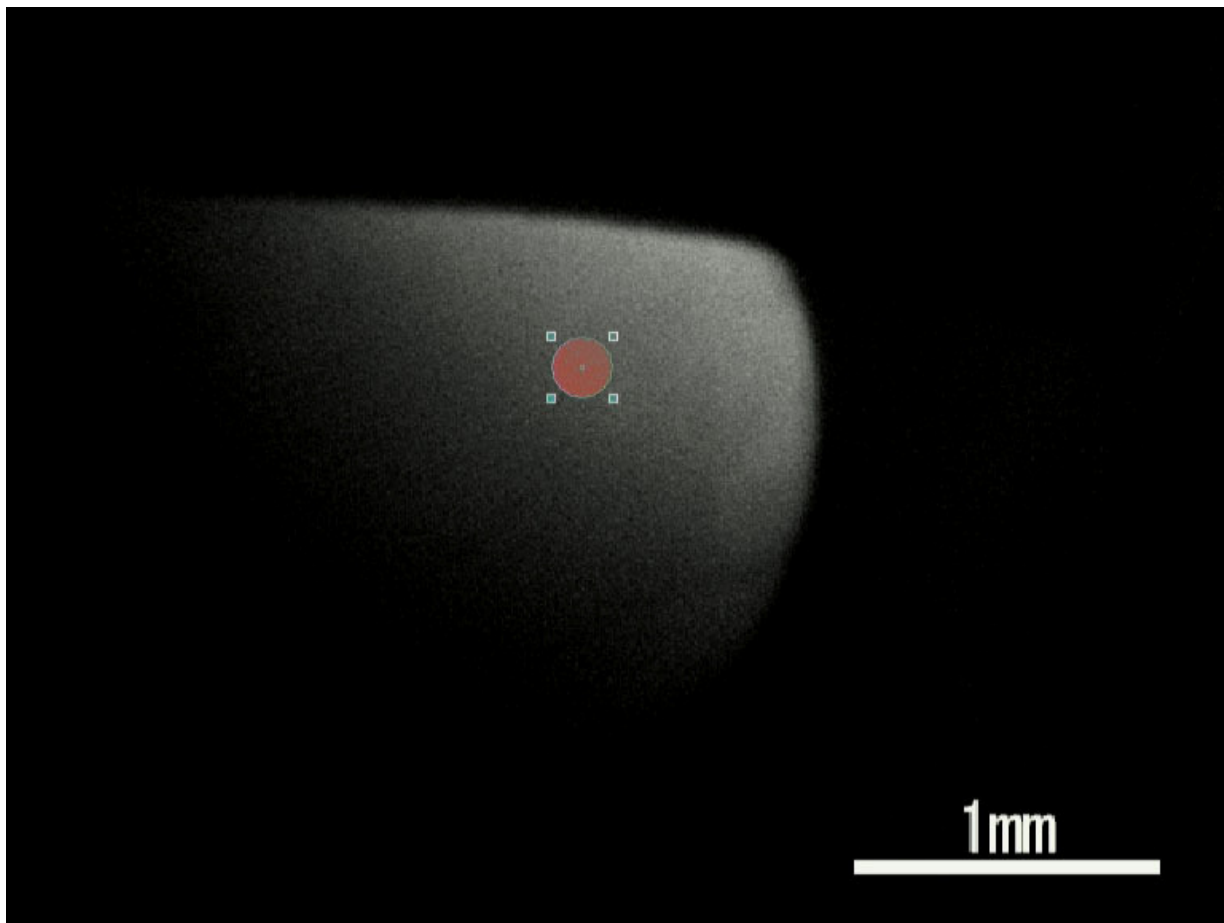**Figure 2.** Two lines showing the X-ray FOV in the vertical position.

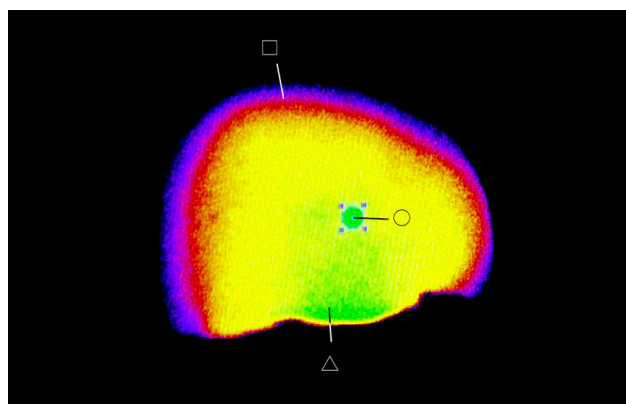**Figure 3.** ROI is composed of 664 pixels in the horizontal position.

Figure 4. Within the yellow-shaded area, a randomly selected ROI (small green circle) is shown. Also shown are beam-hardening (purple outline) and ring (green in the center) artifacts.

and lesions treated with Clinpro Tooth Crème at z1 through z6 (Figure 5). At these slice depths, the mean density values of lesions treated with Clinpro Tooth Crème were significantly higher (p<0.05) as compared to

the baseline WSL. With respect to comparisons between baseline WSL and lesions treated with Clinpro 5000 (Figure 6), the mean density of lesions treated with Clinpro 5000 was significantly higher (p<0.05) than the baseline WSL at z1, z2, z3 and z4 slice depths. Comparisons of mean density values versus slice depth between baseline WSL and lesions treated with Tom's of Maine (Figure 7) revealed the density values were significantly higher (p<0.05) at z1 through z5 for lesions treated with Tom's of Maine.

Density versus slice depth comparison among WSL treated with each of the toothpastes

Comparison of the mean densities at each slice depth for WSL treated with each of the three toothpastes is as shown in Figure 8. At z1, Clinpro Tooth Crème and Clinpro 5000 were not significantly different (p=0.91), but were both superior to the fluoride-free Tom's of Maine (p=0.04 and 0.01, respectively). Similarly, at z2, Clinpro Tooth Crème and Clinpro 5000 were not significantly different (p=0.99) but again were both superior to the
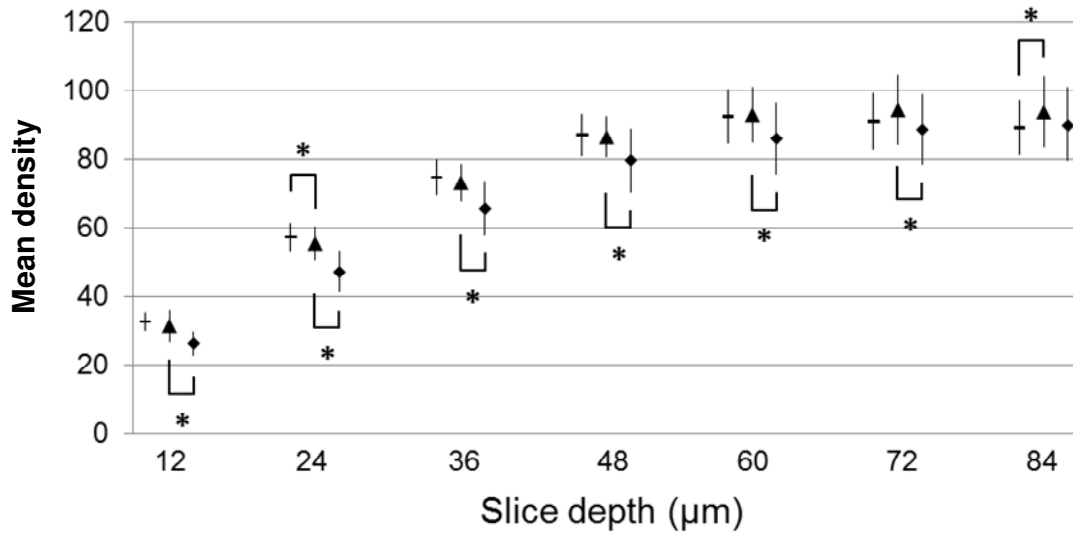
Figure 5. Mean density (standard deviation) versus slice depth for white-spot lesions treated with Clinpro Tooth Crème (B, ▲) and baseline sound enamel (-) and baseline white-spot lesions (♦). Asterisks (*) mark significant differences.
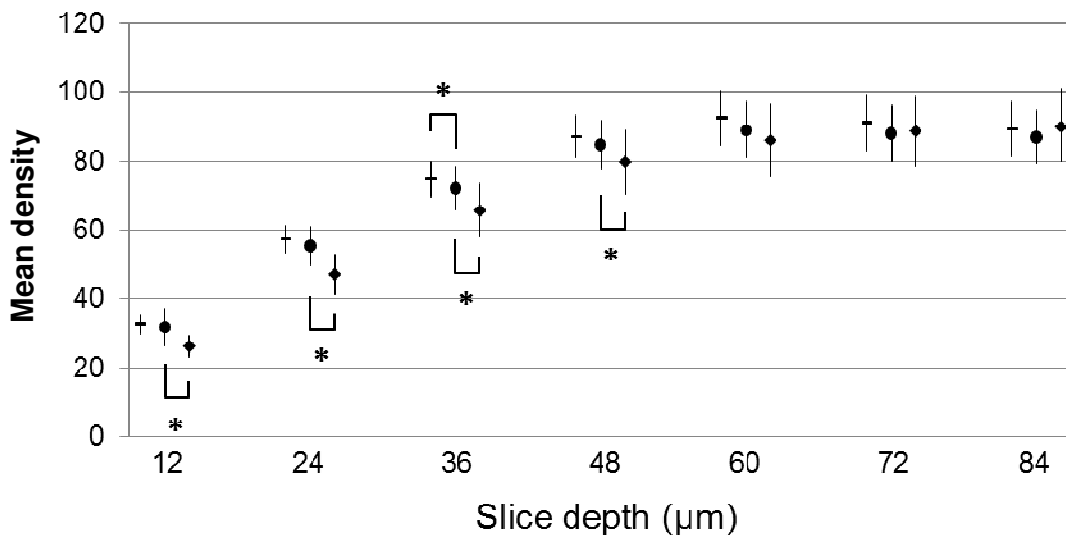

Figure 6. Mean density (standard deviation) versus slice depth for white-spot lesions treated with Clinpro 5000 (C, ●) and baseline sound enamel (-) and baseline white-spot lesions (♦). Asterisks (*) mark significant differences.

fluoride-free Tom's of Maine (p=0.0009 and 0.0004, respectively). At z3, Clinpro Tooth Crème was found to be statistically superior to Tom's of Maine (p=0.01), but statistically similar to Clinpro 5000 (p=0.63), which was not significantly different compared to Tom's of Maine (p=0.08). No significant differences were observed among the three toothpastes at z4 or z5. At z6, Clinpro Tooth Crème was found to be significantly different compared to Clinpro 5000 (p=0.002), but Clinpro Tooth Crème and Clinpro 5000 were not significantly different

compared to Tom's of Maine (p=0.28 and 0.11, respectively). At z7, Clinpro Tooth Crème and Tom's of Maine were not significantly different from one another (p=0.68) but were each significantly different than Clinpro 5000 (p=0.001 and 0.01, respectively).

DISCUSSION

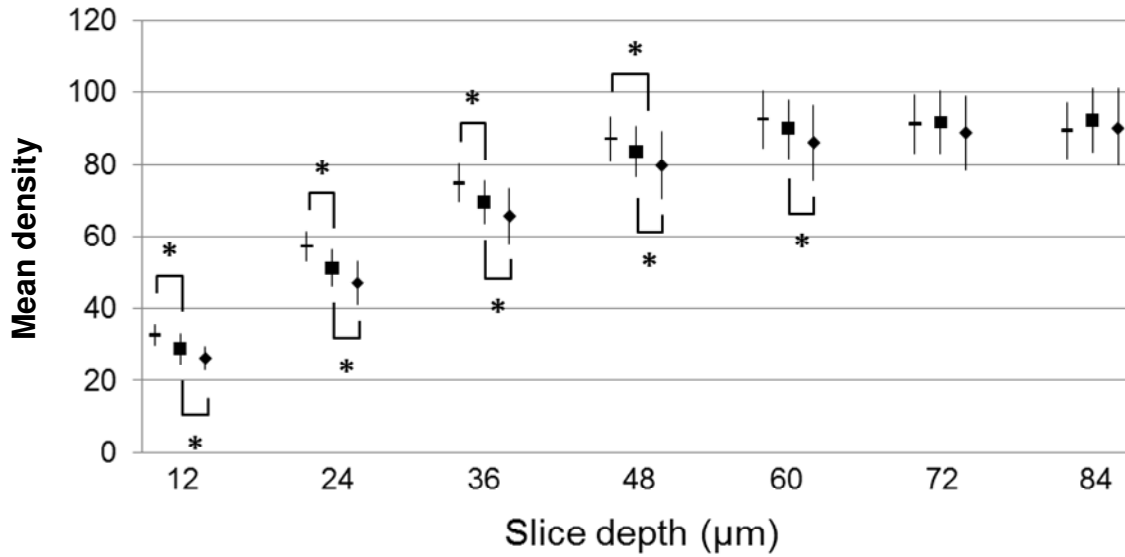This study utilized micro-CT as a tool to characterize the

Figure 7. Mean density (standard deviation) versus slice depth for white-spot lesions treated with Tom's of Maine baseline (A, ■) and baseline sound enamel (-) and baseline white-spot lesions (♦). Asterisks (*) mark significant differences.
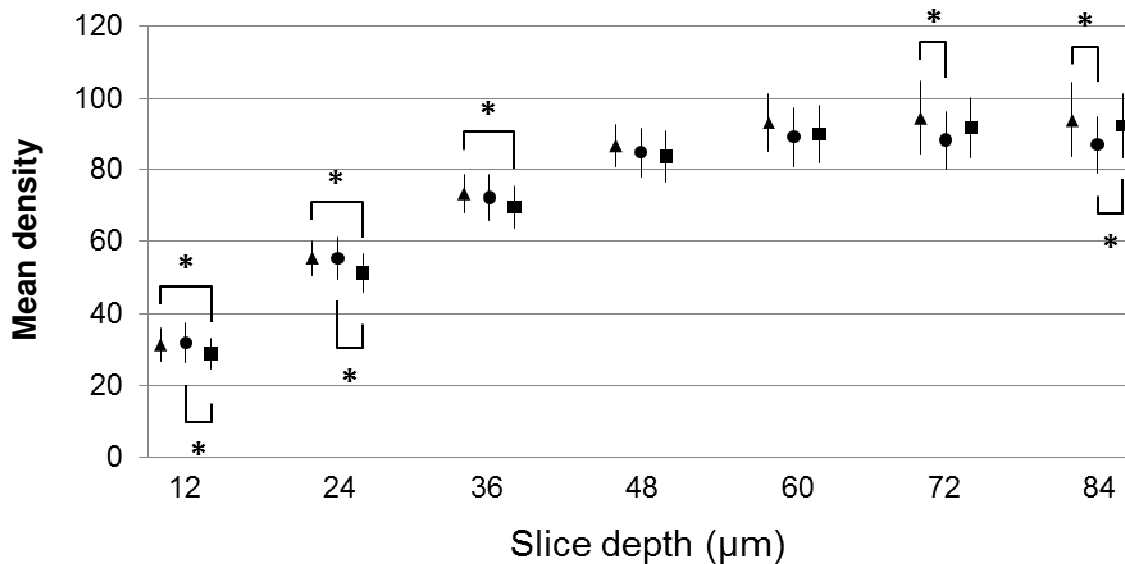

Figure 8. Mean density (standard deviation) versus slice depth for white-spot lesions treated with Tom's of Maine (A, ■) and Clinpro Tooth Crème (B, ▲) and Clinpro5000 (C, ●). Asterisks (*) mark significant differences.

response of incipient lesions subjected to a 10-day pH cycling model manifesting remineralization and demineralization periods and treated with one of three toothpastes. Among the elemental constituents of enamel, calcium is present in the largest concentration (Cate et al., 1988) and is most sensitive to X-ray absorption (Wildenschild et al., 2002). The loss of calcium during initial demineralization of enamel to create the 70 μm-deep lesion accompanies the loss of other enamel constituents as

well, including phosphate, carbonate and hydroxyl groups. Micro-CT has previously been used to probe such compositional changes (Watanabe et al., 2012; Wong et al., 2004). Additionally, the exposure to acid challenges, different toothpaste treatments and remineralization periods in simulated saliva solutions in a pH cycling model leads to chemical and physical changes of the lesion framework (Watanabe et al., 2012).

An important result of this study was that micro-CT

resolved significant differences between either baseline WSL or sound enamel and lesions treated without or with fluoride from three different toothpastes. As shown in Figures 5 to 7, the densities of WSL exposed to the different treatments in the pH cycling model manifest positions between sound and softened (that is, WSL) enamel. With respect to the densities of baseline WSL, each of the toothpastes produced increased densities when evaluated in the 10-day remineralization model. This result demonstrates that the artificial saliva solution encourages remineralization by virtue of its ionic constituents and is consistent with the important role that natural saliva has on the remineralization and/or protection against demineralization of the dentition (Stookey, 2008). But in WSL treated with each of three dentifrices, significant differences were observed as shown in Figure 8. In particular, within the first 25 μm (that is, z_1 and z_2) of the approximately 70 μm-deep lesion, both fluoride toothpastes produced greater densities relative to the fluoride-free Tom's of Maine toothpaste. Because integration of fluoride with the tooth structure improves resistance to demineralization (Karlinsey et al., 2010a it is possible that the lesions treated with the fluoride-free paste succumbed more readily to the daily acid challenges as compared to the two fluoride pastes. Alternately, the lesions treated with fluoride may have increased in density due to the formation of fluoridated mineral such as fluorapatite or calcium fluoride (Cate and Rempt, 1986). Due to the pharmacokinetics of fluoride, its penetration into enamel is limited and decreases exponentially with enamel depth (Stearns, 1970). It may be possible that the functionalized TCP present in the Clinpro dentifrices helps extend the depth of fluoride penetration, and therefore lesion remineralization (Karlinsey et al., 2010d; Mensinkai et al., 2012), as evidenced by Clinpro Tooth Crème having a statistically significant density increase over baseline WSL from 12 to 72 μm. However, high fluoride levels can still restrict penetration depth even with TCP included, considering the Clinpro 5000 group only had statistically significant density increases over baseline WSL from 12 to 48 μm. The data in Figure 8 demonstrate that no significant differences in density among the three toothpastes were observed around 48 μm. Although some differences were observed beyond the bottom of the 70 μm lesion and into sound enamel, these densities are on par with those for sound enamel and it may be possible that the observed differences might be attributed to possible leaching or redistribution of mineral constituents due to penetration of acid during the acid challenge.

Compared with sound enamel at z_2 (that is, ~ 24 μm) no significant differences in WSL density were observed with Clinpro 5000, while the density for Clinpro Tooth Crème was significantly lower. This observation suggests Clinpro 5000, which contains 1.1% NaF, delivers remineralization benefits at the surface of the WSL better than the 0.21% NaF Clinpro Tooth Crème. Since the densities of lesions treated with Clinpro 5000 were similar to those of sound enamel down to 24 μm, this result suggests the rapid reaction kinetics and concentration of fluoride from the high-fluoride dentifrice manifests limitations to the extent of subsurface remineralization relative to a lower fluoride level (that is, Clinpro Tooth Crème). At z_3 (that is, ~ 36 μm) the density of Clinpro 5000 was significantly lower, and trended lower at subsequent depths. This response suggests that high-fluoride Clinpro 5000 may have limited penetration into WSL, a position that is consistent with characteristics of high-fluoride treatments (Tavss et al., 2003), and is also consistent with a previous report on WSL treated with Clinpro 5000 and analyzed using transverse micro-radiography (TMR) (Karlinsey et al., 2011a). And in the absence of fluoride, there exists an increased suscepti-bility for enamel dissolution, which appears to contribute to the markedly lower densities at z_1 through z_4 for lesions treated with fluoride-free Tom's of Maine relative to sound enamel.

The comparison between baseline sound enamel and the WSL treated with each of the pastes was performed to assess the degree to which each toothpaste remineralized incipient enamel lesions. These comparisons suggest fluoride assists in mineral integration, and subsequently affects the bonding environments of enamel constituents, especially with respect to calcium. Based on previous research of surface and subsurface enamel strengthening (Karlinsey and Pfarrer, 2012; Karlinsey et al., 2010b), it is possible that the functionalized TCP ingredient in the Clinpro Tooth Crème and Clinpro 5000 contributes to the observed density measurements; however, additional studies, including those involving fluoride dentifrice without functionalized TCP, would be needed to further understand the nature of these effects. In any case, these observations suggest micro-CT might be useful in resolving effects due to different treatments. As such, studies incorporating different laboratory models that include treatments from dental preparations with various fluoride concentrations and/or other agents could be investigated. Additionally, studies involving lesions of various depths and porosities could be performed to further assess the sensitivity of micro-CT.

Conclusion

Micro-CT was used to assess the remineralization of combination dentifrices having fluoride and functionalized TCP, with both dentifrice systems leading to WSL densities approaching those of sound enamel. Additionally, observations regarding the extent of remineralization with respect to fluoride concentrations were also made. Although the pH cycling model employed in this study has clinical relevance (Karlinsey et al., 2010d; O'Reilly and Featherstone, 1987), a pilot clinical study should be performed to strengthen support for clinical recommendations.

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