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Full Length Research Paper

In vitro assessments of white-spot lesions treated with NaF plus tricalcium phosphate (TCP) toothpastes using synchrotron radiation micro computed tomography (SR micro-CT)

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Synchrotron radiation micro-computed tomography (SR micro-CT), considered superior to standard polychromatic micro-CT techniques, was used to assess the densities of bovine enamel white-spot lesions (WSL) treated in a 10-day pH cycling model with either: (A) Clinpro Tooth Crème (0.21% NaF plus TCP), (B) Clinpro 5000 (1.1% NaF plus TCP) or (C) Tom's of Maine (0% NaF) dentifrice. 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 10 days, WSL specimens were evaluated for lesion depth using confocal microscopy and lesion density using SR micro-CT with depths ranging from 2.76 to 113.16 µm, in 2.76 µm slice increments. Statistical analyses (Student's t-test) were performed at the 95% confidence level. SR micro-CT analyses revealed the NaF plus TCP dentifrices improved WSL densities relative to the fluoride-free toothpaste, and is consistent with an earlier study utilizing polychromatic micro-CT. In contrast to previous findings, SR micro-CT analyses also revealed significant differences in WSL densities treated with the two NaF dentifrices at enamel depths of 13.80, 16.56, and 19.32 µm. These findings suggest SR micro-CT may be especially suited for detecting density differences in lesions sensitive to fluoride-driven remineralization processes.

Key words: Toothpaste, synchrotron radiation micro computed tomography (SR micro-CT), density, remineralization, fluoride, monochromatized X-ray beams, X-ray linear attenuation coefficient (X-ray LAC).

INTRODUCTION

Caries, which is an initially reversible, chronic disease with a known multi-factorial etiology, is being appreciated more widely (Pitts, 2004). Practically, avoiding sugar use and applying perfect oral self-care is difficult to achieve on a population-wide level (Marthaler, 1990). Filling damaged teeth with a restoration should not be considered as an ideal treatment since it does not aim at eliminating the fundamental cause of caries (Elderton, 1996). Protective

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factors such as salivary calcium, phosphate and proteins, salivary flow, fluoride in saliva, and antibacterial agents can balance, prevent or reverse dental caries (Featherstone, 2000; Stookey, 2008). Multiple uses of various fluoride products provide teeth with increased protection against caries (Zimmer et al., 2003). All individuals should use fluoride toothpaste, containing 0.20 to 0.32% NaF, as a basic caries-preventive measure (Kidd and Nyvad, 2003; Twetman et al., 2003). Caries-active patients will need additional fluoride therapy in the form of a home use fluoride mouth-rinses (Marinho et al., 2003b) or professionally-applied fluoride containing products (American Dental Association Council on Scientific Affairs, 2007) until the situation is under the control (Kidd and Nyvad, 2003). In the United States, dental practitioners may recommend professional-strength 1.1% sodium fluoride toothpastes for high-risk patients instead of the typical range found in fluoride toothpastes (e.g. 0.20 to 0.32% NaF) that are 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 of approximately 70 and 22%, respectively (Tavss et al., 2003). Such therapies might be recommended for various at-risk dental populations, such as orthodontic patients. The inherent cleaning difficulties that directly result from 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 is comprised of β–tricalcium phosphate and sodium lauryl sulfate, to NaF formulations (Karlinsey and Pfarrer, 2012). In particular, 3M 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 functionalized TCP ingredient in NaF formulations has been shown to produce stronger, more acid-resistant mineral relative to fluoride alone in laboratory and clinical evaluations (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 conventional micro computed tomography (micro-CT) which utilizes polychromatic X-rays. CT is a non-destructive method that provides cross-sectional images (CT images) of objects using X-ray attenuation. A conventional micro-CT has been used in the observation of time-course changes in remineralization (Nakata et al., 2012), demineralization (Watanabe et al., 2012) and remineralization of white-spot lesions (WSL) treated with NaF plus TCP systems (Asaizumi et al., 2013). In these experiments, a CT image is expressed as a spatial distribution of so-called CT values, which correspond to the X-ray linear attenuation coefficient (X-ray LAC) of a material obtained by tomographic reconstruction. Three-dimensional (3-D) internal structures can subsequently be obtained by stacking successive CT images. Though these images provided significant insight into a material's density, a potential drawback in using conventional micro-CT instruments is the fact that these systems can give rise to method-derived artifacts that may influence the accuracy of the data. One way to help improve image quality, and therefore the 'true' characteristics of the material, could be through the use of a synchrotron radiation source with well-characterized theory-experiment X-ray LAC relationships. A synchrotron radiation (SR) source provides tunable, monochromatized, and naturally collimated (parallel) X-ray beams that have many advantages for CT (Flannery et al., 1987; Bonse and Busch, 1996). Monochromatized beams eliminate beam hardening effects, which causes CT image artifacts (Figure 1), and thus permit CT values to relate quantitatively to X-ray LACs. Furthermore, collimated beams readily yield 3-D images with high spatial resolution. Hirano et al. (1990) applied a SR microtomographic system at the Photon Factory in Japan to a meteorite sample, although they were unable to obtain 3-D images, because they used a linear X-ray detector. Fortunately, a quantitative relation between the X-ray LAC obtained by Synchrotron Radiation X-ray computed tomography (known as 'observed X-ray LAC') and the theoretically calculated X-ray LAC (known as 'theoretical X-ray LAC') of standard materials (such as minerals and metals) has been obtained using an X-ray microtomographic system at BL20B2 of the SPring-8 facility in Japan. This system, called SP-μCT, uses highly monochromatized and well-collimated partially coherent X-ray beams produced by a synchrotron radiation source. Uesugi et al. (1999, 2010) have developed an X-ray microtomographic system, named SP-μCT, using SR at SPring-8. The present method for calibrating CT values or observed X-ray LAC is applicable to any X-ray CT system that utilizes monochromatic beams (Tsuchiyama et al., 2005).

In our previous endeavors, utilizing a conventional micro-CT at the Advanced Composite Technology Center, which is part of the Japan Aerospace Exploration Agency (JAXA) (Asaizumi et al., 2013), no significant differences were found in the densities of enamel lesions treated with either the 0.21 or 1.1% NaF dentifrices containing TCP (Figure 2). But because these dentifrices contained markedly different fluoride content which in turn bears on clinical efficacy (Tavss et al., 2003), it may be possible that the conventional micro-CT instrument was not sensitive enough to distinguish fluoride-treated lesions due to its inherent experimental limitations as noted earlier. In order to rule out this possibility while improving our understanding of these two fluoridated dentifrice

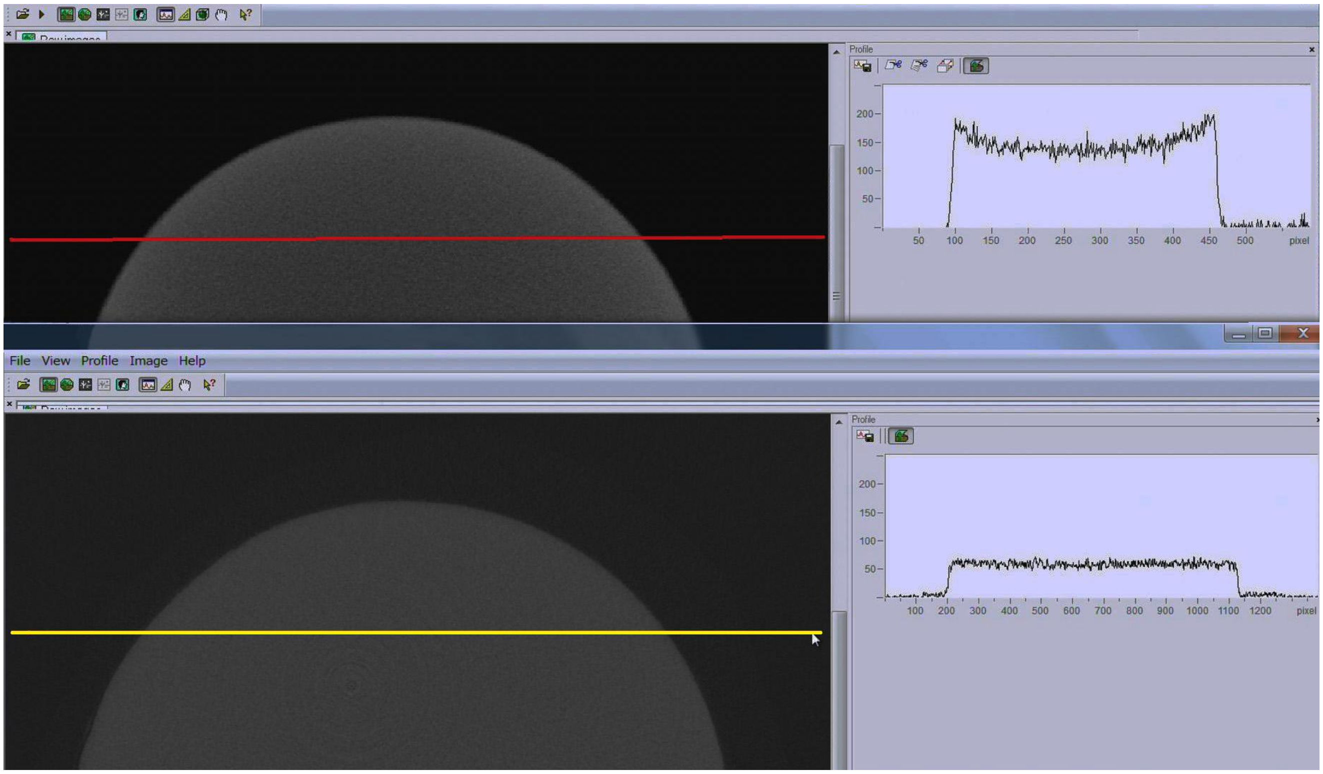

Figure 1. An example view of the signal profile comparison shown between the conventional micro-computed tomography (micro-CT) which utilizes polychromatic X-ray beams (top image with red line) and the synchrotron radiation micro-computed tomography (SR micro-CT) employed at SPring-8 which utilizes monochromatic X-ray beams (bottom image, with yellow line). In the top image, beam hardening effects were observed at the edges of the enamel specimen, giving rise to a non-linear signal profile. In the bottom image, the beam hardening effects were not as pronounced, with signal providing a more stable and flat character. Specimen No. 517 from the Tom's of Maine treatment group was used for this comparison.

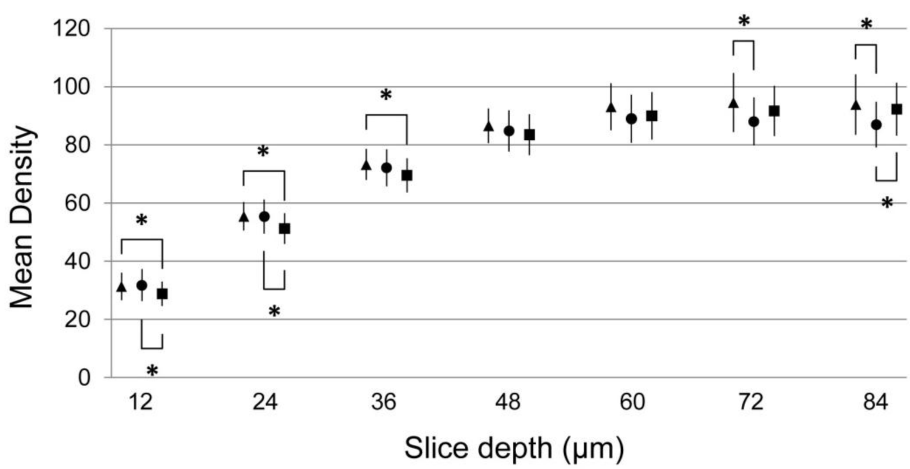

Figure 2. These data were obtained in our prior experiment at JAXA (Asaizumi et al., 2013). Mean density (standard deviation) versus slice depth for white-spot lesions treated with and Clinpro Tooth Crème (A, ▲), Clinpro 5000 (B, ●) and Tom's of Maine (C, ■). Asterisks (*) mark significant differences.

systems, the purpose of this follow-on study was to use SR micro-CT to further assess the characteristic densities of WSL previously treated with a fluoride-free dentifrice, along with those treated with either the 0.21% NaF plus

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

Event	Duration
Treatment 1*	2 min
Saliva, pH =7.0	1 h
Treatment 2	2 min
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 the first day, specimens were immersed in artificial saliva for one hour prior to the first treatment.

TCP (Clinpro Tooth Crème) or 1.1% NaF plus TCP (Clinpro 5000) dentifrice. To help frame our assessments, evaluations of control 'sound' and 'lesioned' enamel specimens were also included. It is our understanding of the literature that SR micro-CT has only been used to measure the X-ray LAC between sound and carious enamel (Dowker et al., 2004). Therefore, in addition to our interests in better understanding evaluations of fluoride-sensitive lesions, this application of SR micro-CT on WSL enamel exposed to different fluoride concentrations serves as a first approach in the study of enamel remineralization.

MATERIALS AND METHODS

Treatment groups and study protocol

Since this study was a follow-on to the previous laboratory study and utilized the same enamel specimens as used previously, full experimental details are not discussed here, but are summarized only and can be found in greater detail in our prior publication (Asaizumi et al., 2013). The same bovine enamel specimens (N=10 per group) as examined previously were maintained in their respective groups as follows: (A) Clinpro Tooth Crème (0.21% NaF plus TCP); (B) Clinpro 5000 (1.1% NaF plus TCP); (C) Tom's of Maine (fluoride-free toothpaste). SR micro-CT was also performed for control purposes with sound (N=10) and WSL (N=10) specimens. 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 4 h polyacrylic acid-lactic acid challenge (15 ml, pH=5.0), and finally two more 2 min 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). Each specimen was evaluated for WSL depth using a digital microscope (VHX-2000, KEYENCE Corporation, Japan) before performing SR micro-CT.

SR micro-CT

SR micro-CT was used for density measurements at SPring-8 in Hyogo, Japan. The parameters used to collect SR micro-CT data, which were optimized based on the best image collected, are shown in Table 2. The standard parameters filter function was used to probe densities of specimens. An example view of the SR micro-CT apparatus and the mounted specimen fixed to the turn table are as shown in Figure 3. From this setup, an example 'field of view' (FOV) image was taken, as shown in Figure 4. The 3-D data from the 50 bovine enamel specimens (N=10 for each treatment groups, plus 10 baseline sound and 10 baseline WSL) were collected as distributions of X-ray LAC.

Calibration

Four glass capillaries comprising H_2O, K_2HPO_4 (22.07 and 45.83 weight percentage) aqueous solution and SiO_2 (silica glass) were used to obtain the X-ray LAC correction curves. The same size region of interest (ROI) with bovine enamel was used to measure the X-ray LAC at the center of the glass capillary for each sample (Figure 5). The mean values of the four groups were analyzed.

Analysis

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. Five ROIs were randomly selected from one slice picture (Figure 5). ROI was based on the surface from the top-view perspective as shown in Figure 5. Since the bovine enamel surfaces were not perfectly flat, a small ROI helped to reduce inclinations at the top surface. In doing so, the possibility of involving ring artifacts in the ROIs was reduced and careful consideration was used in selecting ROIs lacking these types of artifacts. Densities were then determined at each slice depth ranging from 2.76 to 113.16 µm, with 2.76 µm slice thickness. The sound enamel control group had one specimen that produced X-rays out of range of the FOV. The Clinpro 5000 group had a specimen manifesting resins on the surface. Because of these specimens, only nine of the ten specimens in the sound enamel control group and only nine of the ten in the Clinpro 5000 group provided data and were measured at 40 places with 54 slice depths per place for a total of 2160 ROIs. Otherwise, all ten specimens from each of the other two toothpaste groups, along with the WSL control group, were measured at 50 places with 54 slice depths per place for a total of 2700 ROIs.

Digital microscopy analysis

Each of the 50 specimens (10 from each group) was measured 20 times for lesion depth in cross-sectional view as shown in Figures 6 and 7.

Statistics

SR micro-CT

All statistics were determined using the statistical package SAS-JMP (SAS Institute, USA). The mean densities at each depth (2.76 µm through 113.16 µm) for Clinpro Tooth Crème and Clinpro 5000 were defined as independent variables. Each measurement was considered of equivalent variance so parametric testing (Student's t-test) of the mean densities between Clinpro Tooth Crème and

Table 2. Exposure parameters comparison of two kinds of micro-CTs.

Place	JAXA	SPring-8
Scan date	May-29-2012	May-22-2013
Scan time (s)	355.8	450.0
Recon. time (s)	5.7	90.0
Conventional micro-CT tube voltage	90 kV	-
SR micro-CT energy	-	30 keV
number of views	800	1500
Integration number	3	1
FOV (mm)	6.987	5.652
Slice thickness (mm)	0.012	0.00276
System name	TOSCANER-30000	SP-μCT
Matrix size	1024	2048
Scan mode	180°	180°
Filter function	Laks	Ramachandran
Data mode	Cone beam	Parallel beam

Table 3. X-ray LAC correction curves were obtained from calculated LACs and observed LACs of H_2O, K_2HPO_4 (22.07 and 45.83 weight percentage) aqueous solution and SiO_2 (silica glass).

Parameter	H_2O	K_2HPO_4 (22.07 wt%)	K_2HPO_4 (45.83 wt%)	SiO_2
LAC at 30 keV	0.38	0.88	1.60	1.91
Density (g/cm^3)	1.00	1.20	1.46	2.22
LAC at exp	0.35	0.79	1.31	1.77
SD of LAC	0.0072	0.0068	0.0079	0.0067

wt%: Weight percentage.

Clinpro 5000 was performed, which was the purpose of this study. Data was normally distributed.

Digital microscopy

Lesion depths (μm) were measured from cross-sectional views using Keyence VHX-2000 operating software on WSL treated with Clinpro Tooth Crème, Clinpro 5000, Tom's of Maine (fluoride-free control) and untreated WSL (control). Lesion depths were randomly selected and measured 20 times by each sample (n=20×10). Comparisons between the lesion depth mean values for the three toothpaste groups and untreated WSL group were performed via analysis of variance (ANOVA, Welch test).

RESULTS

SR micro-CT

Calibration

CT values in the present study, or X-ray LAC derived from tomographic reconstructions, are called 'observed X-ray LAC', whereas theoretically calculated X-ray LAC will be called 'theoretical X-ray LAC'. In this study, the quantitative relation between observed and theoretical X-ray LACs has been obtained by imaging standard mineral materials in Figure 8 (Tsuchiyama et al., 2005).

SR micro-CT

Mean density versus slice thickness distance comparisons with Clinpro Tooth Crème and Clinpro 5000

Comparison of the mean densities at each slice depth for WSL treated with two toothpastes is shown in Figure 9. Comparisons of the mean densities at each slice depth for WSL treated with either Clinpro Tooth Crème or Clinpro 5000 were made. At 13.80, 16.56 and 19.32 μm, Clinpro 5000 was statistically significantly higher than Clinpro Tooth Crème ($p<0.05$) using Student's t-test (Figure 9).

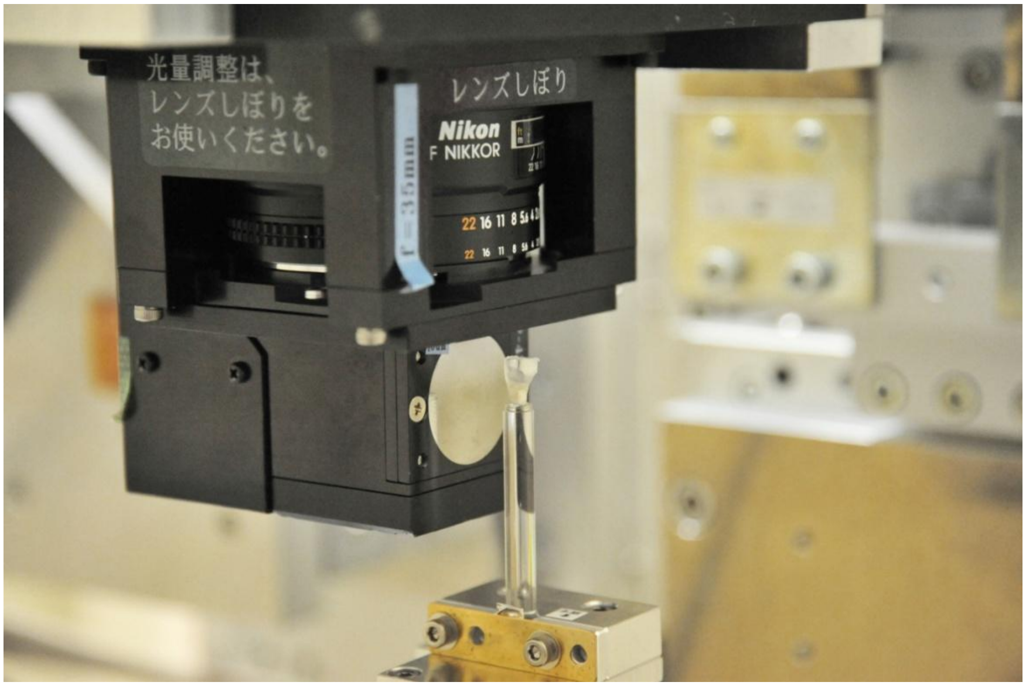

Figure 3. Representative view of an enamel specimen positioned on the rotary stage in the Experimental hutch 1.

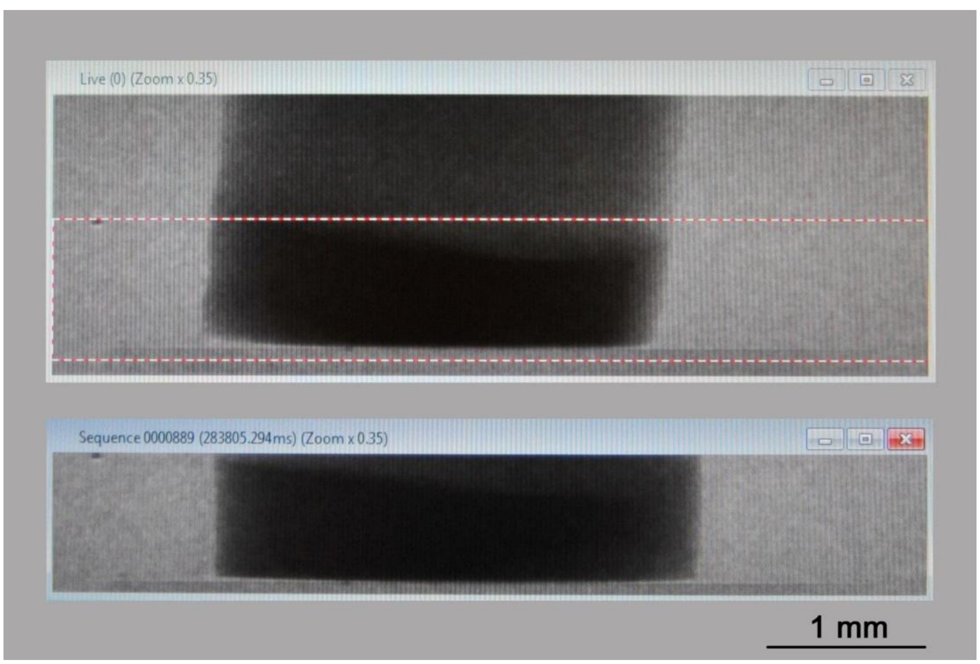

Figure 4. Two red dotted lines showing the X-ray FOV in the vertical position. Bovine enamel was set upside down on an acrylic rod using pure water to settle.

Digital microscopy

ANOVA (Welch test) was performed for lesion depth analysis of enamel specimens treated with Clinpro Tooth Crème, Clinpro 5000 and Tom's of Maine. For reference, untreated enamel WSLs were also included. Significant differences were not found in this lesion depth comparison.

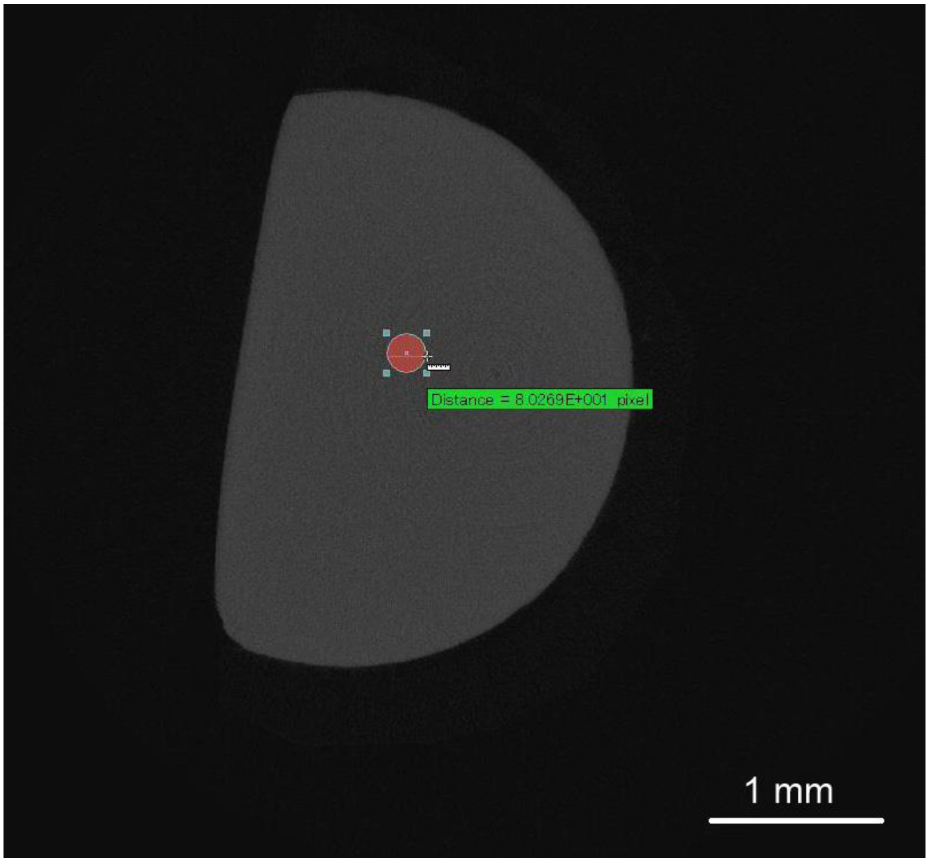

Figure 5. An example horizontal view of an enamel specimen. The ROI (red circle) is composed of 6969 pixels. It corresponds to 222 μm in diameter. 4 glass capillaries comprising H_2O, K_2HPO_4 (22.07 and 45.83 weight percent) aqueous solution and SiO_2 (silica glass) also measured the same ROI size to obtain the X-ray LAC correction curves.

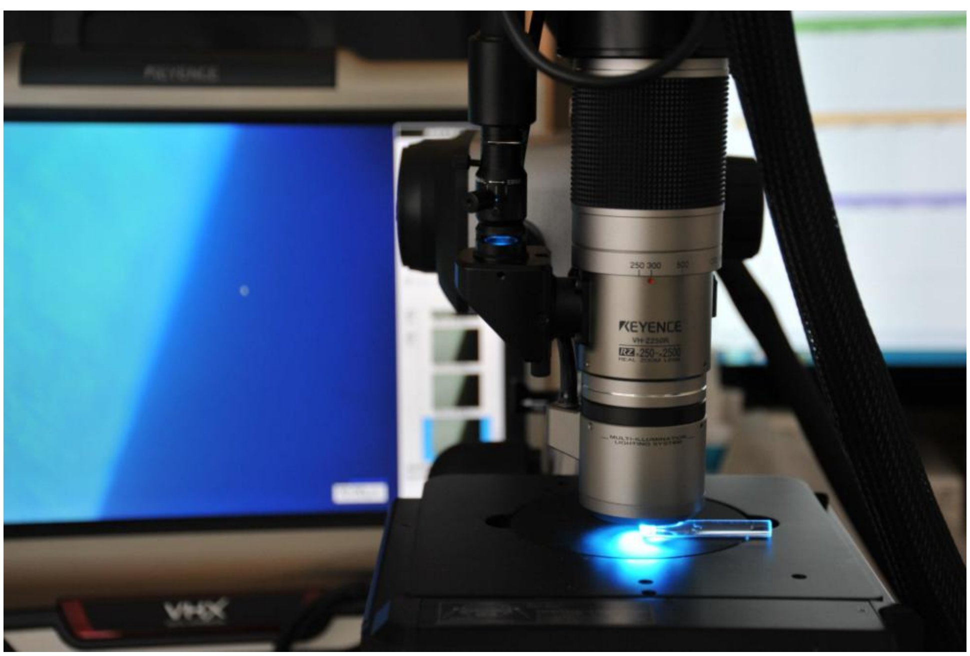

Figure 6. An example setup of digital microscopy measurement in cross-sectional view.

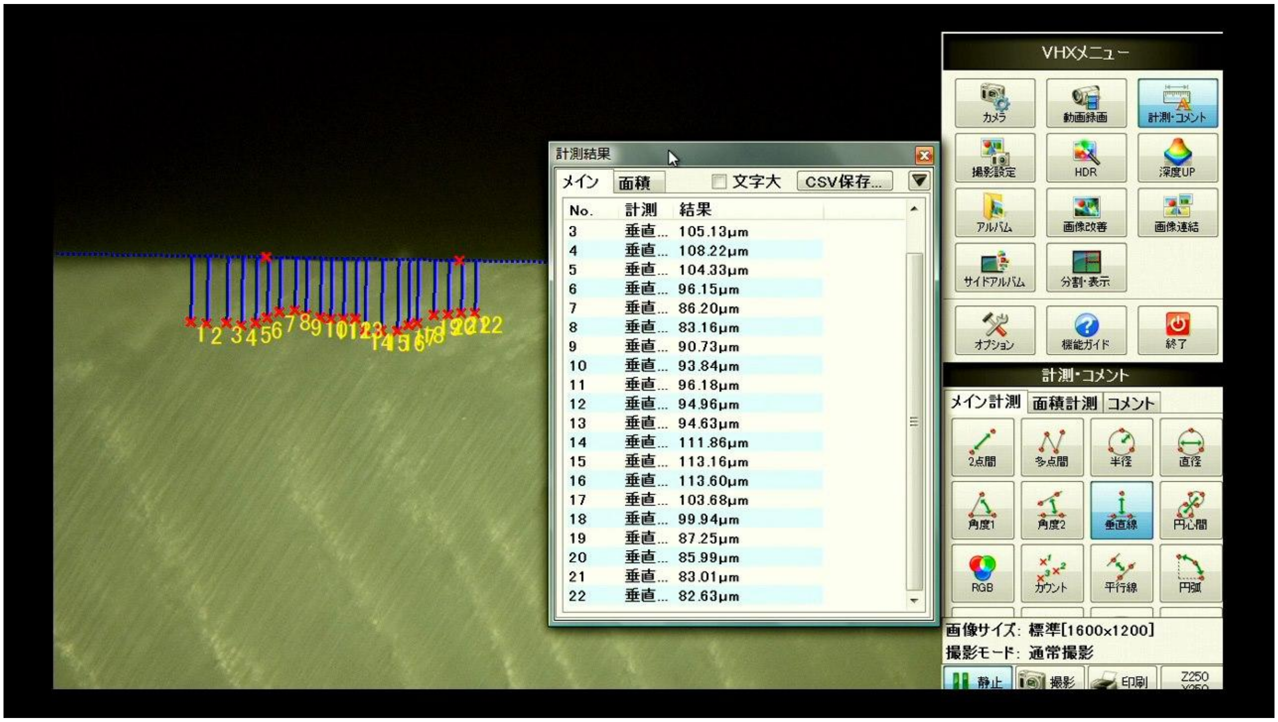

Figure 7. Lesion depth (µm) was randomly measured 20 times using digital microscopy operating software for WSL specimens treated with Clinpro Tooth Crème, Clinpro 5000, fluoride-free Control Tom's of Maine and no treatment (Control WSL).

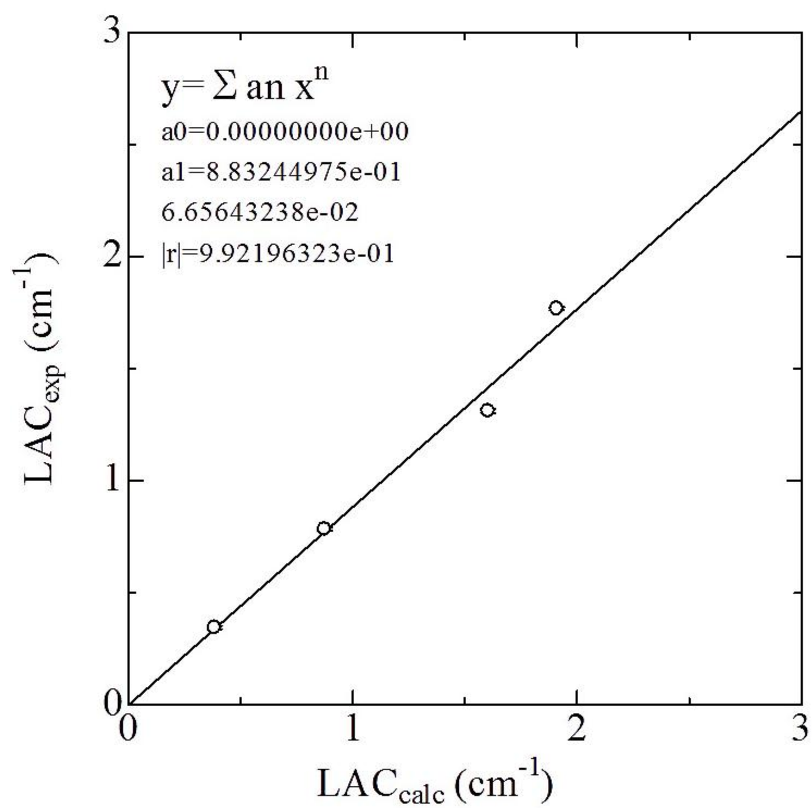

Figure 8. A representative calibration curve. The relationship between the measured value and calculated value was 0.883.

DISCUSSION

This study utilized SR micro-CT to further characterize the response of incipient lesions subjected to a 10-day pH cycling model manifesting remineralization and demineralization periods and treated with one of three dentifrices. In our prior work (Asaizumi et al., 2013), no significant differences were observed in WSL treated with either Clinpro Tooth Crème or Clinpro 5000, despite the relatively large difference in fluoride concentration (0.21% NaF versus 1.1% NaF). It is reasonable that the nature of the experimental method used, which incorporated polychromatic X-rays and spatial resolution, could have obscured potential differences. In an attempt to better understand the limitations of conventional micro-CT, as well as further probe the effect of fluoride concentration on lesion depth, we repeated the prior study on the same enamel specimens using a monochromatic-based SR micro-CT method.

Monochromatic X-rays are used in synchrotron radiation and can eliminate beam hardening effects, which can inadvertently obscure 'true' density measurements of the material under examination. This is important, since the relationship between the CT value and X-ray LAC is not straight forward in almost all of the conventional micro-CT where polychromatic X-ray beams are used (Figure 1). This is mainly due to the beam hardening effects, where the energy distribution spectra of the beams are modified through absorption as lower energy X-rays are absorbed more strongly than higher-energy ones (Denison et al., 1997).

It was noted that although microscopy provided measurements of lesion depths near 70 μm, which was observed different lesion depths via SR micro-CT, as observed in Figures 11, and this may be due to the defined 'start point' (or 'sample surface'), where X-rays impinge on the first slice of bovine enamel. These differences are evidenced, for instance, in Figures 9, 10 and 11, where it was noted that at a microscopy-based lesion depth near 70 μm, the SR micro-CT-based densities continue to increase until they plateau around 113.16 μm from the sample surface. That there exists a difference in these lesions depths based on microscopy versus SR micro-CT methods might be based on two explanations. First, the lesion depth obtained by the digital microscope may not clearly demarcate the boundary line edge since this is primarily based on visual observation. However, if the boundary is clearly visible, then it is reasonable that some degree of demineralization extends beyond the boundary line. Such effects, which are likely too subtle to be captured visually, may appear quite similar to sound enamel.

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 microscopy-based 70 μm-deep lesion accompanies the loss of other enamel constituents as well, including phosphate, carbonate and hydroxyl groups (Figure 11). Additionally, the exposure to acid challenges, different toothpastes 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), all of which bear on the density determined by conventional micro-CT (Watanabe et al., 2012; Wong et al., 2004). Here, it was observed that enamel lesions treated with fluoride may have become denser due to the formation of fluoridated mineral such as fluorapatite or calcium fluoride (Cate and Rempt, 1986). These apparent density increases extend into the body of the enamel lesions and contrast markedly with enamel treated with the fluoride-free dentifrice.

An important result of this study was that SR micro-CT resolved statistically significant differences between different concentrations of two fluoridated toothpastes (0.21% NaF and 1.1% NaF). Comparisons between Clinpro 5000 and Clinpro Tooth Crème revealed significant differences at 13.80, 16.56 and 19.32 μm. These observations suggest 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. These results further suggest application of high fluoride concentrations may inherently limit the extent of subsurface remineralization relative to a lower fluoride concentration. Beyond 19.32 μm, the densities of WSL treated with Clinpro 5000 trended lower. This response suggests that the 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). Interestingly, as observed in the trending patterns in Figures 9, 10 and 11, Clinpro Tooth Crème appears to improve WSL density over the entire lesion depth, as well as into the relatively sound enamel region, where it is consistent with expectations of sound enamel (Figure 9). This is an interesting result that may speak to the effects of a 0.21% NaF dentifrice. As it relates to the Clinpro 5000 dentifrice, the lower concentration of fluoride in Clinpro Tooth Crème may provide mineralizing benefits that help sustain the action of fluoride throughout the body of the lesion to protect against possible leaching from beyond the lesion-sound enamel boundary. These comparisons suggest fluoride assists in mineral integration, and subsequently affects the bonding environments of enamel constituents, especially with respect to calcium. Since penetration of fluoride into enamel is limited and decreases exponentially with enamel depth (Stearns, 1970), it might 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). This view

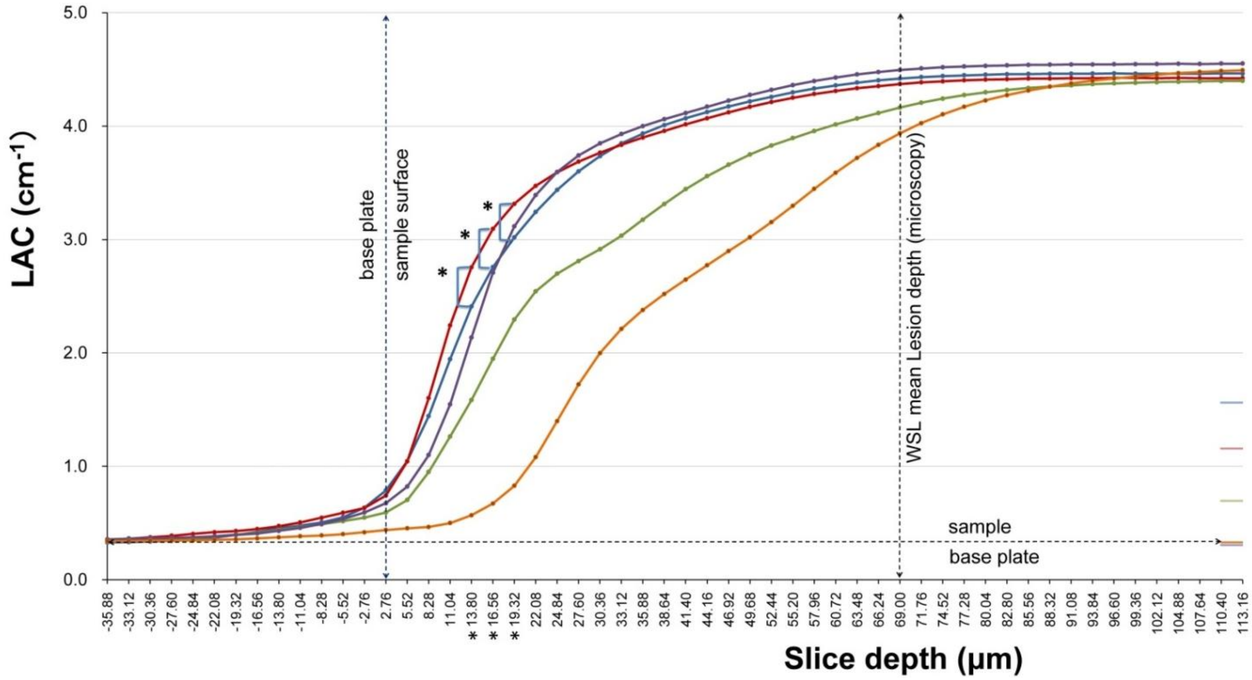

Figure 9. X-ray LAC (cm^{-1}) versus slice depth (μm) comparisons with Clinpro Tooth Crème (blue) and Clinpro 5000 (red). Images of bovine enamels were obtained with 30 keV X-rays. X-ray LAC (cm^{-1}) versus slice depth (μm) for White-spot Lesions treated with Clinpro Tooth Crème (blue) and Clinpro 5000 (red). Asterisks (*) mark significant differences. Control Tom's of Maine (green), Control Sound Enamel (purple) and Control White-spot Lesions (orange) are shown as references. An edge-enhanced contrast due to X-ray propagation was used to superpose five groups and to define the first slice depth with respect to the enamel-air interface (Wilkins et al., 1996; Momose, 2005).

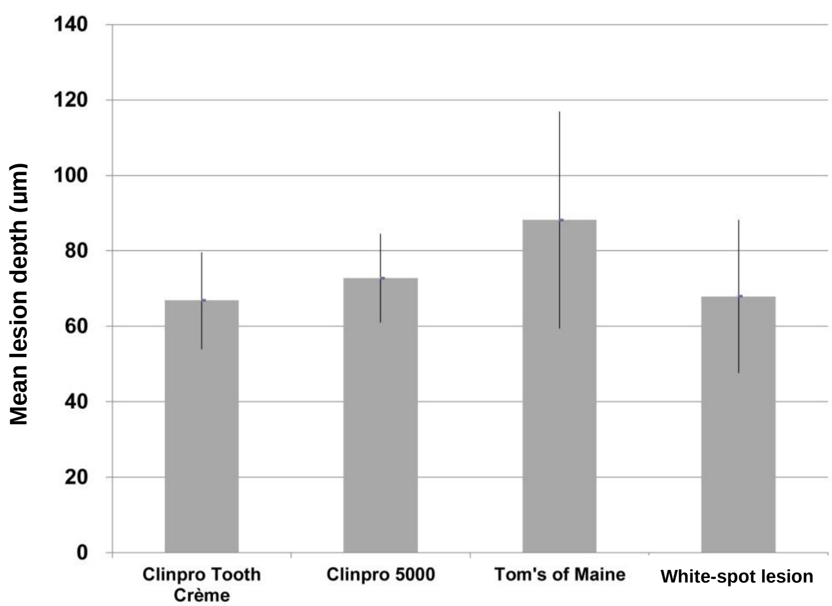

Figure 10. Lesion depth comparison for WSL treated with Clinpro Tooth Crème, Clinpro 5000, Control Tom’s of Maine and Control White-spot Lesions (F-value=1.572, df=2, p=0.2285, ANOVA Welch test). Black bars indicate standard deviation.

is suggested based on previous research of surface and subsurface enamel strengthening (Karlinsey and Pfarrer, 2012; Karlinsey et al., 2010b) and further examinations with non-TCP dentifrices, including evaluations of enamel

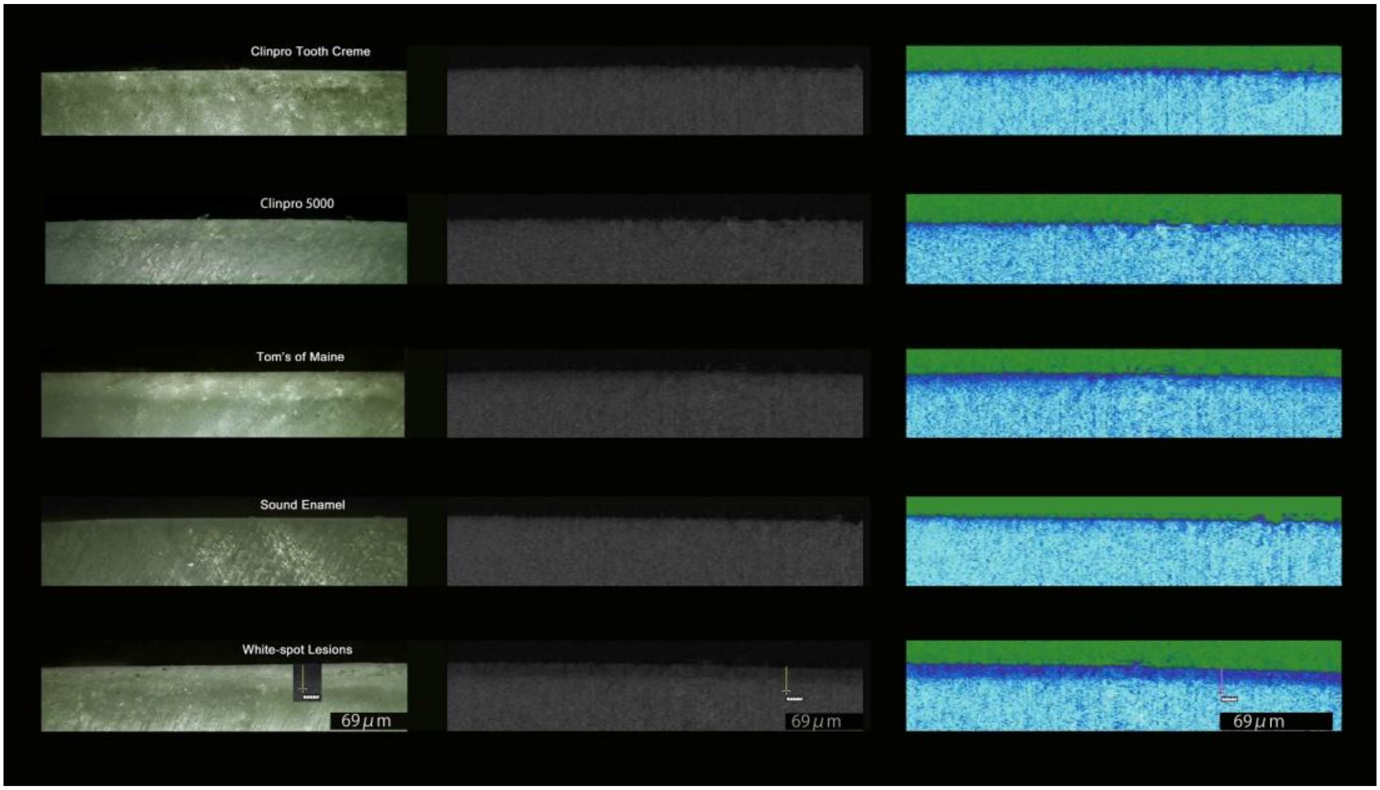

Figure 11. From the top to the bottom shows cross-sectional views of an enamel sample selected randomly for each treatment group of Clinpro Tooth Crème, Clinpro 5000, Tom's of Maine, Sound Enamel, and White-spot Lesions (microscopy on the left, SR micro-CT original view in the middle, and color filtered SR micro-CT used to visualize the lesion depth on the right).

specimens subjected to *in situ* clinical studies, are recommended to further understand these effects.

But the central thesis of this study suggests SR micro-CT can be useful in resolving the subtle fluoride-sensitive mineralizing effects of enamel lesions. Presumably, this SR micro-CT may also be used to assess other promising mineralizing systems. We note that since many of the marketed toothpastes comprise a range of mineralizing agents it may be possible to identify density differences in enamel based on toothpaste constituency. 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 SR micro-CT.

Conclusion

SR micro-CT was used to assess the remineralization of dentifrices having two different fluoride concentrations, with both dentifrice systems leading to WSL densities approaching those of sound enamel. It is our understanding that this is the first attempt at using SR micro-CT in the investigations of enamel lesions treated with different fluoride concentrations. 'Observed X-ray LAC' and 'Theoretical X-ray LAC' relation was obtained in this study. Additionally, observations regarding the extent of remineralization with respect to fluoride concentrations were also made, with the identification of significant differences in lesions treated with either 0.21% NaF or 1.1% NaF. These findings are unique in that prior studies using polychromatic X-ray beams were unable to distinguish such differences. Therefore, in order to better assess subtle differences in remineralized (or demineralized) enamel, it may be particularly insightful to use SR micro-CT.

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