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# Journal of Dentistry and Oral Hygiene

Table of Content: Volume 6 Number 1 January 2014

## ARTICLES

### Research Articles

**Dental care practices and caries experiences among teenagers in Hhohho sub-region, Swaziland** 1

S. L. Mndzebele and O. N. Makhubela-Nkondo

***In vitro* assessments of white-spot lesions treated with NaF plus tricalcium phosphate (TCP) toothpastes using synchrotron radiation micro computed tomography (SR micro-CT)** 10

Makoto Asaizumi, Kentaro Uesugi, Masato Hoshino, Tomoaki Kato, Allen C. Mackey and Robert L. Karlinsey



Full Length Research Paper

## Dental care practices and caries experiences among teenagers in Hhohho sub-region, Swaziland

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Increased figures in tooth extractions among the youth population in the Northern Hhohho region of Swaziland prompted the researchers to investigate these development, with the purpose of assessing and describing dental healthcare practices and caries experiences among school going teenagers. The study engaged a randomized-survey design through a self-administered questionnaire. The population sample comprised 562 secondary school-going pupils (13 to 18 years). On self-reported caries experiences, about 43.3% reported to have had one or more decayed-teeth; 21.9% were found to be consuming foods comprising refined-carbohydrates daily; and about 30.3% often go to bed chewing sweets. About 98.8% brush their teeth at least once a day, and about 77.8% knew other unconventional tooth brushing methods. Logistic regression results suggest that pupils who were within 10 km of a dental clinic were 30% less likely to be found with decayed-teeth ( $e^B=0.719$ ;  $p\text{-value}=0.082$ ). Surprisingly, pupils who knew the cause of caries were almost twice likely to be found with decayed-teeth ( $e^B=1.866$ ;  $p\text{-value}=0.003$ ). Those who took soft-drinks for their daily refreshments increased their chances of being found with decayed-teeth by three-folds ( $e^B=3.33$ ;  $p\text{-value}=0.008$ ). Pupils who knew the difference between tooth-decay and gum disease were twice likely to be found with decayed-teeth ( $e^B=1.918$ ;  $p\text{-value}=0.003$ ). It can therefore be concluded that whilst caries affect teenagers uniformly (whether boys/girls); most of the known caries causal factors do not seem to influence the probability of having caries in this region.

**Key words:** Dental caries experiences, oral hygiene, dietary habits, tooth-brushing, teenagers.

### INTRODUCTION

Some personal oral healthcare practices and perceptions that often have a direct influence on caries development such as frequency of tooth-brushing and certain dietary habits continue to occupy a central role in the field of oral health research today. For instance, it is important to understand the role played by even a simple fruit juice in caries development. That is why caries has now been considered to be the major oral health problems throughout the world (Evans et al., 2013; Ministry of Health and Social Welfare, 2005; Marthaler et al., 1996; Petersen, 2003). With gum-diseases on its side, caries has been noted as significant in societies by the World Health

Organization (WHO), because almost everybody has experienced one or more of these two conditions at some point in time (WHO, 2001). Caries is related to one's lifestyle, and behavioural factors under a person's control are clearly implicated. These factors include poor oral hygiene; poor dietary habits, that is, frequent consumption of refined carbohydrates, frequent use of oral medications that contain sugar, and inappropriate methods of feeding infants (Selwitz et al., 2007).

There is strong evidence to the effect that certain foodstuff is the main cause of dental caries in most people (Rugg-Gunn and Nunn, 1999; WHO, 2006; Rees, 1992).

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Rees (1992) claims that adolescent individuals often choose foods high in fat and sugar that consist of fast-foods and ready-to-eat snack foods which are known causes of caries on teeth. Further, while Sheiham (2001) concurs with the aforementioned argument of a strong association between sugar and dental caries, he argues that there is no such relation between starch and caries. He claims that individuals who eat low sugar diets, but high starch would normally experience low caries levels. Above all arguments, the bottom line is that children are the most vulnerable group to dental caries. The vulnerability of teenagers to caries also varies with socio-economic groupings and geographical locations. For instance, Gugushe and du Plessis (1998) in a study to understand the regional and countrywide urban-rural distribution of dental caries in Swaziland concluded that in developing countries, children from higher socio-economic groups often residing in the urban areas have more dental decay than children from lower socio-economic groups residing in rural areas. Although Radford et al. (2000) conducted their study on infants, the socio-economic background of their subjects suggest that individuals living in areas of highest deprivation had higher frequencies of caries compared to those from more affluent areas. This is another example of how some individuals are more vulnerable to caries compared to others.

The Swaziland government through the Ministry of Health and Social Welfare (1994) noted that there was no significant increase in the experience of dental caries in 12-year old children. However, there was a big difference in caries experiences in the same age cohort between rural and urban areas with a 'decayed-missing-filled-teeth' (DMFT) of 0.64 for the rural and 1.23 for the urban areas. For periodontal diseases, the prevalence of gingivitis was 100% whilst that of calculus was also discovered to be very high for an age cohort of a 15 year old (Ministry of Health and Social Welfare, 1994). Four years later, Gugushe and du Plessis (1998) investigated the regional urban-rural distribution of dental caries in Swaziland amongst 6 to 44 years old. The two researchers discovered that in the Lubombo region, the dental caries experience of 6 and 12 years children was significantly higher in the urban areas than in rural areas. For the Shiselweni region, only the 12 years old had a significant difference  $p=0.0018$  in the urban-rural caries experience. There was no significant difference  $p>0.05$  observed in the urban-rural dental caries experience of all the other age groups and the regions (Gugushe and du Plessis, 1998). Due to insufficient empirical data on oral health in Swaziland, the worse in oral health conditions is likely to have occurred in the years.

In addition to the oral hygiene and diet, there are other social and geographical factors that put teenagers at risk to dental caries. This was noted by Höllund (1990) and the International Food Information Council (1998). A study by Höllund (1990) whose purpose was to examine

the structure of attitudes towards healthy food in a group of adolescents, and evaluate the impact of a health education programme on these attitudes; she concluded that the change in attitudes can be explained by two mechanisms: (1) dissonance arousal and discrepancy between personal attitudes and group norms; (2) and that future programmes should emphasise affection rather than cognition (Höllund, 1990). Similarly, the International Food Information Council (1998) argues that lack of good rules on oral health by individuals as well as good nutrition would normally put their oral health at high risk. In some instances, both social and geographical factors may either provide unfavourable or favourable conditions in the form of accessibility to efficient health care services. The aim and purpose of this cross-sectional study was therefore, to assess and describe the dental care practices and caries experiences of teenagers in the Northern Hhohho region of Swaziland. The key focus was on their personal oral hygiene practices, dietary habits, distances and visits to the dental clinic, oral healthcare perceptions, which all somehow informed their caries experiences.

## MATERIALS AND METHODS

This study engaged a randomised survey design. The University of South Africa Ethics Committee approved the study. Permission was also sort from the Regional Education Office and from the governing bodies of the selected schools. Following a pre-test of the tool to improve validity and reliability, some minor adjustments were made on certain questions for clear understanding. A total of 527 schools going teenagers (13 to 18 years) were targeted in the Northern Hhohho region of Swaziland through a two-stage random sampling. At first, we selected the specific schools which were distributed between rural and urban areas, and then went on to select the actual classes within these schools through a simple random sampling method. This resulted to a total of 10 schools (5-rural and 5-urban schools) being selected. In each selected class, all consented pupils participated in the study as their age limit did qualify them by law to give their own consent for participating in the study. Data collection was through self-administered questionnaires as (in the case of caries) the study did not aim to measure the actual DMFT prevalence of the pupils, but reference was on caries occurrences through self-reported caries experiences. Prior arrangements were made with the schools such that within a period of two weeks all the schools would have been completed. The main categories per the structuring of the assessment tool were: caries occurrence, oral hygiene practices, dietary intake, and visits to the dental clinic which were all assessed using the self-administered questionnaire. The last question comprised of an open-ended statement on their views on how to prevent caries among teenagers. In each class, after a brief description of the study purpose and how to go about in filling the questionnaire, consented pupils were given the questionnaire to fill. When all had finished, all questionnaires were collected and placed in the respective envelopes.

## Data analysis

Following data cleaning, we were then left with a total of 508 usable responses for data analysis. Data were captured and analysed using

**Table 1.** Respondents' dietary habits.

Variable	Frequency	Proportion (%)
<b>Eat sweetened foods</b>		
Always	18	3.5
Sometimes	402	79.1
Never	88	17.2
Total	508	100
<b>Snacking with</b>		
Sweets	57	11.2
Fruits	308	60.6
Chips	111	21.9
Other	31	6.3
Total	508	100
<b>Lunch-box pack</b>		
Bread and juice	295	58.1
Cookies and juice	34	6.7
Fruits and juice	88	17.3
Other	91	17.9
Total	508	100

the Statistical Package for the Social Sciences (SPSS-16.0). The t test, odds ratio calculations and the chi-square test (at a level of significance of  $p=0.05$ ) were used during the preliminary analysis. Logistic regression analysis was also conducted to assess the bivariate relationship between the presence or absence of self-reported decayed-teeth by the pupils (the response variable) and each of the nineteen explanatory variables which included: sex/gender of the pupil, the age, whether they were boarders or day scholars, whether they lived with at least one of their parents, whether they had a tooth brush or not, whether the pupil's gums bled when brushing teeth or not, whether the pupil brushed teeth two times or more per day, whether they knew of other (traditional) methods of cleaning teeth, whether the pupil could list all carbohydrates foods found in the community and could tell which ones were known for causing tooth decay, whether the pupil was in the habit of eating sweetened food during spare time after lunch, whether their daily snack consisted of sweets or not; whether their daily drink consisted of soft drinks or not, whether the pupil sometimes went to bed chewing a sweet or a "chappies", whether it was easy to buy sweets from the school tuck-shop or not, whether their lunch box normally consisted of cookies and juice, whether the pupil could tell the difference between tooth decay and gum disease, whether the distance to the nearest dental clinic was more than 10 km or not, and whether the pupil ever visited the dental clinic to check their teeth at least once a year. At the first stage of the binary logistic regression, all the 19 independent variables in the model were included, and then an automatic forward-selection method was employed in which variables with the greatest significance were added one at a time. Lastly, an automatic back-ward selection procedure was employed. The logistic regression equation that was employed was of the form:

$$\log_e(P/(1-P)) = \text{Const} + B_1X_1 + B_2X_2 + \dots + B_kX_k + u].$$

## RESULTS

### Descriptive differentiations

Based on the target and the final total of 508 usable

responses for analysis, 96.3% response rate was discovered. A proportion of 66.3% of the respondents were within the stipulated age-cohort of 13 to 18 years as some were 12 years and others were 19 years. In terms of gender, 55.1% were boys whilst 44.9% were girls. On whether a respondent was a boarder or a day-scholar, it was found that 8.3% were boarders whilst 91.7% were day-scholars. In terms of owning tooth brushes, 94.1% of the respondents claimed to have toothbrushes. Out of those who owned toothbrushes, 98.8% brushed their teeth at least once a day; whilst a proportion of about 77.8% knew other unconventional tooth brushing methods. On the overall, it was found that about 23.3% of the respondents do regular dental checkups at least once a year. About 28.3% of the pupils reported bleeding gums during tooth brushing. A proportion of about 63.8% claimed to know the difference between gum diseases and caries. About 11.2% of the respondents claimed to be snacking with sweets, whilst 59.6% snacked with fruits. It was also found that about 6.7% of the respondents often pack their school lunch-boxes with sweet-cookies, whilst 17.3% use fruits and juices. A proportion of about 30.3% of the pupils often go to bed chewing sweets. Further, a proportion of about 87.4% of the respondents claimed they could list carbohydrates foods that cause caries as demonstrated in Table 1. In the case of tooth decay experiences, as many as 43.3% of the respondents indicated through their responses to have been currently living with one or more decayed teeth, whilst 71.9% knew how a tooth starts to decay. This figure is only based on reported present-caries, not filled or removed teeth. Only 23.2% of the respondents mentioned that they undertook dental check-ups at least once a year.

### Test for level of significance

Although some of the resultant p-values were not significant at  $p=0.05$  following the binary logistic regression analysis on the number of self-reported decayed-teeth and most of the 19 explanatory variables; the following relationships were noted as demonstrated in Tables 2 and 3: (1) the relationship between the response variable (number of self-reported decayed-teeth) and tooth-brushing frequency was such that  $0.05 < p < 0.10$ ; (2) the relationship between the number of self-reported decayed-teeth and the respondent's gender was such that  $0.10 < p < 0.25$ ; (3) the relationship between the number of self-reported decayed-teeth and going to bed chewing sweets was such that  $0.10 < p < 0.25$ ; (4) the relationship between the number of self-reported decayed-teeth and the use of friends and tuck-shops as key sources of sweets at school was such that  $0.25 < p < 0.75$ ; (5) the relationship between the number of self-reported decayed-teeth and distances away from the dental-clinic was such that  $0.25 < p < 0.75$ ; (6) finally, the relationship between the number of self-reported

**Table 2.** Squared differences between the observed and the expected values on having decayed teeth against some of the explanatory variables.

Variable	Number of carious teeth					T/T
	3 or more	2 Teeth	1 Tooth	None	Not sure	
<b>Squared differences between the observed and the expected values on number of carious teeth per mouth based on the frequency of tooth brushing, divided by (E) expected</b>						
3 times daily	4.94	1.04	0.00	3.05	0.00	9.03
2 times daily	2.36	0.97	0.00	1.01	0.15	4.49
Once daily	0.17	0.40	0.02	0.00	0.37	0.96
Totals	7.47	2.41	0.02	4.06	0.52	14.48
<b>Squared differences between the observed and the expected values on number of carious teeth per mouth based on sex, divided by (E) expected</b>						
Males	0.00	1.14	0.04	0.96	0.63	2.77
Females	0.00	1.40	0.05	1.18	0.37	3.00
Totals	0.00	2.54	0.09	2.14	1.00	5.77
<b>Squared differences between the observed and the expected values on number of carious teeth per mouth based on going to bed chewing sweets, divided by (E) expected</b>						
Chew-sweets (yes)	0.70	0.06	1.57	0.04	0.50	2.87
Chew-sweets (no)	0.30	0.03	0.68	0.02	0.21	1.24
Totals	1.00	0.09	2.25	0.06	0.71	4.11
<b>Squared differences between the observed and the expected values number of carious teeth per mouth against whether a child is a boarder or a day-scholar, divided by (E) expected</b>						
Boarder	0.22	0.38	1.64	0.50	1.28	4.02
Day-scholar	0.02	0.03	0.14	0.04	0.11	0.34
Totals	0.24	0.41	1.78	0.54	1.39	4.36

**Table 3.** Squared differences between the observed and the expected values on having decayed teeth against some of the explanatory variables.

Variable	Having decayed teeth		
	Yes	No	Totals
<b>Squared differences between the observed and the expected values on having decayed teeth against sources of sweets within the school environment, divided by (E) expected</b>			
Friends	0.17	0.22	0.39
Teachers/Vendors	0.00	0.00	0.00
Tuck-shops	0.31	0.40	0.71
Totals	0.48	0.62	1.10

Table 3. Contd.

<b>Squared differences between the observed and the expected values on having decayed teeth against distances from the dental clinic, divided by (E) expected:</b>			
>50 km away	0.01	0.01	0.02
10-49 km away	0.24	0.32	0.56
<10 km away	0.06	0.08	0.14
Totals	0.31	0.41	0.72

decayed-teeth and whether a pupil was a day-scholar or boarder at the school was such that  $0.25 < p < 0.75$ . These findings imply that all hypothetical beliefs in regard to these relationships may be confirmed as such, as per the fact that the resultant p-values were not statistically significant at  $p=0.05$ .

### Model diagnostics results

The examination of each of the nineteen independent variables gave an indication of how it is likely to be associated with the response variable (the presence or absence of dental caries) in a binary regression model. The results from the analysis as demonstrated in Tables 4 and 5 seemed to suggest the following: (1) more than a half of respondents had at least one tooth missing or decayed or filled (54.9%) as against 45.1%; (2) most of the 19 independent variables were not significantly associated with the absence or presence of dental carries, and this was shown by the test z-statistics and their corresponding p-values (the z-statistics were used to test the equality of two proportions in two independent samples – and a normal approximation was used because of large samples); (3) the only variables that showed statistical significance were: taking soft drinks (p-value = 0.009) and having knowledge of traditional methods of cleaning teeth (p-value=0.006). However, knowing the causes of dental caries was associated with the presence or absence of dental caries in a totally unexpected direction, in that the respondents who had this knowledge had a significantly higher proportion of individuals with dental caries (as compared to those who did not claim any such knowledge). Therefore, from the earlier results, it would be expected that the binary logistic model would not yield many statistically significant regression coefficients.

## DISCUSSION

### Dental caries experiences amongst teenagers

This study results confirm previous research findings in respect to the strong correlation between certain dietary habits and the occurrence of caries among the youth population group within the HHohho region. The results

are consistent with the caries status as indicated in the hospital statistics, as per the revelations that more than half of the respondents had at least one tooth decayed or missing or filled (54.9% as against 45.1%), and this demonstrated the fact that dental problems among school-going children in Northern Hhohho region of Swaziland were very serious. Whilst the study did not aim to determine the DMFT prevalence in the 13 to 18 years old group within the mentioned region; previous studies (Ministry of Health and Social Welfare Report, 1994) noted that there was no significant increase in the experience of dental caries in 12 years old children in Swaziland. In the current study, emphasis is on caries occurrences as a result of self-reported caries experiences by the respondents including their dietary habits and oral hygiene practices. For dental caries to develop on the tooth you have to have certain types of bacteria present in the oral cavity, and the individual has to consume refined sugars frequently (WHO, 2001; van der Hoeven and van Palenstein Helderma, 1998; van Palenstein Helderma et al., 1996; Chestnutt and Gibson, 2002). So a continuous interaction over time between these factors would lead to caries development resulting from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms (Selwitz et al., 2007).

A proportion of 56.9% of the pupils were found to be getting sweets from friends within the school premises, whilst 34.6% bought sweets from tuck-shops at school. However, on the issue of having decayed teeth against these key sources of sweets (friends, vendors, tuck-shops) within the school environment, the significance test proved that such sources do not have a strong association with caries, as the  $\chi^2$  value of 1.10 was such that  $0.25 < p < 0.75$ . Implying that there is no specific source of sweets within the school environment that can be attached to caries development among the pupils, rather than the continuous interactions between bacteria and other host in the mouth factors. Here, we need to focus on the risk factors of developing caries. For instance, if we are saying about 21.9% of the pupils consume cakes or cookies almost daily (as per the findings), based on the frequency and amount, this dietary habit exposes them to high risk of caries development. Support of this claim was derived from a study conducted amongst 11 to 14 years old English pupils investigating the correlation

**Table 4.** Relationships between the 19 explanatory variables and having a decayed tooth.

Explanatory variable	Values of the explanatory variable	Dental caries? (dependent variable)		Total	Percent "Yes"	z-statistic and p-value for testing equality in % "yes"
		No	Yes			
Sex	Female=0	95	129	224	57.6	z=1.118, p=0.264 <sup>ns</sup>
	Male=1	131	146	277	52.7	
Type of scholar	Day scholar=0	202	257	459	56.0	z=1.633, p=0.102 <sup>ns</sup>
	Border=1	24	18	42	42.9	
Living with whom	No parents=0	78	110	188	58.5	z=1.263, p=0.207 <sup>ns</sup>
	With at least one parent=1	148	165	313	52.7	
Having tooth brush	No=0	14	15	29	51.7	z=-0.368, p=0.713 <sup>ns</sup>
	Yes=1	211	260	471	55.2	
Bleed in gums when brushing	No=0	161	195	356	54.8	z=-0.242, p=0.809 <sup>ns</sup>
	Yes=1	62	79	141	56.0	
Know traditional methods	No=0	49	64	113	56.6	z=0.414, p=0.679 <sup>ns</sup>
	Yes=1	177	211	388	54.4	
Can list carbohydrates	No=0	65	71	136	52.2	z=-0.74, p=0.459 <sup>ns</sup>
	Yes=1	160	203	363	55.9	
Know decay-causing foods	No=0	26	37	63	58.7	z=0.656, p=0.512 <sup>ns</sup>
	Yes=1	200	238	438	54.3	
Take sweet food > lunch	No=0	44	43	87	49.4	z=-1.107, p=0.268 <sup>ns</sup>
	Yes=1	182	231	413	55.9	
My daily snacks=sweets	No=0	201	243	444	54.7	z=-0.099, p=0.921 <sup>ns</sup>
	Yes=1	25	31	56	55.4	
Cookies or cakes daily	No=0	183	208	391	53.2	z=-1.434, p=0.152 <sup>ns</sup>
	Yes=1	43	67	110	60.9	
My daily drink is soft drinks	No=0	219	251	470	53.4	z=-2.601, p=0.0093****
	Yes=1	7	24	31	77.4	

Table 4. Contd.

Gone to bed chewing sweets?	Never=0	151	198	349	56.7	z=1.241, p=0.215 <sup>ns</sup>
	Sometimes=1	75	77	152	50.7	
Tuckshop is easiest source of sweets	No=0	143	185	328	56.5	z=0.941, p=0.347 <sup>ns</sup>
	Yes=1	83	90	173	52.0	
Cookies/juice lunch box	No=0	212	256	468	54.7	z=-0.324, p=0.746 <sup>ns</sup>
	Yes=1	14	19	33	57.6	
Know how t-decay starts?	No=0	77	63	140	45.0	z=-2.744, p=0.006 <sup>***</sup>
	Yes=1	149	211	360	58.6	
Can tell t-decay from gum disease?	No=0	83	95	178	53.4	z=-0.581, p=0.561 <sup>ns</sup>
	Yes=1	141	180	321	56.1	
Nearest dental clinic	>10k (=0)	128	172	300	57.3	z=1.345, p=0.179 <sup>ns</sup>
	≤10k (=1)	98	103	201	51.2	
Visit to dental clinic	Never=0	174	209	383	54.6	z=-0.190, p=0.849
	At least once=1	52	65	117	55.6	

correlation between the frequency/weight of intake of dietary items high in sugars. The conclusions drawn from the earlier study was that foods such as: sugared-tea, sweet hot drinks, sweet pudding, soft drinks, chocolate, biscuits, and cakes have a high correlation-coefficient( $r$ ) of +0.80 to +0.98 (Sheiham, 2001). Similarly, for those pupils who either take soft-drinks or eat sweets regularly (11.4 and 11.2%, respectively); the correlation-coefficient between the frequency and weight for tea is believed to be +0.98, whilst for sweets, it is believed to be +0.74 (Sheiham, 2001). This again may suggest that about 11.4% of the pupils in the current study may be at a high risk of developing caries. Even the results that followed the binary regression revealed similar chances in that those

who took soft-drinks for their daily refreshments were three times likely ( $e^B=3.33$ ;  $p=0.008$ ) to being found with dental caries when compared with those who chose other types of drink such as water and juice. Of note, there seemed to have had a negative regression coefficient which pointed to the wrong direction on going to bed eating sweets, which seemed to have reduced the odds of getting dental caries to 40% ( $e^B=0.647$ ;  $p=0.045$ ). Also, knowing what causes tooth decay seemed to double the likelihood of getting dental caries ( $e^B=1.918$ ;  $p=0.003$ ), although this one seemed to be a contrast.

Some of the schools in Swaziland have enrolled both day-scholars and boarders. This situation cannot be overlooked when looking at dental care

practices and caries experiences amongst teenagers. In the case of caries occurrences between day-scholars and boarders, the resultant p-value was found to be not statistically significant in that the  $\chi^2$  value of 4.36 was such that  $0.25 < p < 0.75$ , implying that the number of carious teeth per individual cannot be determined by whether a pupil is a boarder or day-scholar, thereby confirming any hypothetical belief in this regard. On another note, pupils having decayed teeth against distances away from the dental clinic, the findings were that the  $\chi^2$  value of 0.72 was such that  $0.25 < p < 0.75$ , this implies that being far-away or closer to the clinic has no effect on caries experiences. However, the logistic regression results suggest the opposite in that pupils who were within

**Table 5.** Results of the three variable-selection procedures.

<b>Method 1: All 19 variables included</b>	<b>B</b>	<b>p-value<sup>1</sup></b>	<b>e<sup>B</sup></b>
Constant	1.452	0.234	4.272
Sex	-0.188	0.349	0.828
Type of scholar (day or boarding)	-0.479	0.185	0.619
Whether pupil was staying with parents	-0.181	0.360	0.834
Ownership of toothbrush	0.121	0.771	1.128
Bleeding of gums when brushing teeth	0.166	0.451	1.181
Knowledge of other traditional methods	-0.220	0.341	0.802
Knowing all carbohydrate foods	0.133	0.556	1.142
Knowing which foods cause tooth decay	-0.490	0.111	0.613
Taking sweetened food after lunch	0.231	0.361	1.260
Carrying snacks of sweets daily	-0.020	0.947	0.980
Daily drink being soft drinks	1.189	0.012**	3.283
Sometimes going to bed with sweets	-0.435	0.045**	0.647
School tuckshop gives easy access to sweets	-0.214	0.342	0.807
Lunch box is made up of cookies/juices	-0.157	0.697	0.855
Eating cookies or cakes everyday	0.240	0.323	1.271
Knowledge of what causes tooth decay	0.651	0.003***	1.918
Can tell difference between tooth decay and gum disease	0.058	0.784	1.060
Distance to nearest dental clinic being within 10 km	-0.295	0.134	0.744
Visiting the dental clinic at least once a year	0.054	0.821	1.056
<b>Method 2: Forward selection method</b>			
Constant	-0.294	0.098*	0.745
Daily drink being soft drinks	1.122	0.013**	3.071
Knowledge of what causes tooth decay	0.619	0.003***	1.857
<b>Method 3: Backward selection method</b>			
Constant	-0.168	0.381	0.845
Daily drink being soft drinks	1.205	0.008***	3.338
Knowledge of what causes tooth decay	0.624	0.003***	1.866
Distance to nearest dental clinic being within 10 km	-0.330	0.082*	0.719

<sup>1</sup>One star \*Indicates significance at the 10% level, \*\*Indicate significance at the 5% level, \*\*\* Indicate significance at the 1% level.

pupils who were within 10 km of a dental clinic significantly reduced the odds by 30% of being found with caries ( $e^B=0.719$ ;  $p=0.082$ ) compared to pupils who lived more than 10 km away. The findings suggest that there is a need for health authorities to design oral health services that are universal, and that do not differentiate between pupils in terms of type of schooling (boarders or day-scholars), race, color, or creed.

It can therefore be concluded that, whilst all the other suggested explanatory variables were not statistically significant, it indicates to a large extent that the high caries occurrences found in the Northern region of Swaziland affects children alike. The study suggests that caries affect pupils uniformly, whether they are young or older; whether they are boarders or day-scholars; whether their gums bleed while brushing their teeth or not; whether they have knowledge of carbohydrates foods that

that cause teeth to decay or not; whether they retire to bed chewing sweets or not; whether they snack with cookies or sweets; whether they visit the dental clinic at least once a year or not. In other words, these mentioned factors did not seem to affect the pupil's probability of having dental caries.

### **Influence on oral hygiene practices among teenagers**

A proportion (78.7%) of the pupils claims to have been brushing their teeth either once or twice daily. This points to the fact that during most part of the day, they are at school, suggesting that there are currently serious limitations or unavailability of school dental health programs in most schools in the country. School dental health programs are the key in motivating children towards regular



tooth brushing both during school hours and at home. The primary measures in preventing dental diseases involve the maintenance of a good oral hygiene (Oregon Health and Science University, 2006; American Dental Association, 2004; International Food Information Council, 1998). Amongst boys, there was a consistent decrease in the proportion of pupils brushing their teeth frequently; whilst there was a concomitant increase in the proportion of girls brushing their teeth. In addition, the fact that the frequency of bleeding gums decreased with increased frequency of toothbrush possession does suggest regular use of a toothbrush to confirm ownership. Although when we ran the significance test on the number of carious teeth/mouth against the frequency of tooth brushing, the value was not statistically significant in that the  $\chi^2$  value (14.48) was such that  $0.05 < p < 0.10$ , thereby confirming any hypothetical belief in this regard. The test was only statistically significant at 10%, implying that the frequency of tooth brushing by individuals does have an effect on the number of carious teeth/mouth/an individual. These revelation calls for better strategies in the promotion of tooth brushing programs at all school levels.

Some schools in Swaziland are predominately rural, hence it is by no surprise that a proportion of 77.8% of the pupils claimed that they knew unconventional tooth cleansing devices. These may include certain types of traditional tooth brushing devices such as the use of certain chewing-sticks, roots of certain plants, and charcoal amongst others. Although this behaviour could not influence much of the caries experiences amongst many individuals as has been revealed through the statistical test. For instance when we tested the significance level on having decayed teeth (number) against knowledge of unconventional tooth brushing devices such as chewing-sticks and in others, it was found to be statistically not significant in that the  $\chi^2$  value of 4.58 was such that  $0.25 < p < 0.75$ . This suggests that having knowledge of some traditional tooth brushing devices or methods does not determine the number of carious teeth in an individual. However, further statistical analysis through the use of the binary regression, revealed that having knowledge of these traditional methods against having or not having decayed teeth was statistically significant ( $p=0.006$ ). Although traditional tooth cleansing were the best alternatives in the past; nowadays, most people seem not interested in these methods. They consider chewing-sticks as too primitive.

The primary strengths of this study were the diverse differences between rural and urban locations of the selected schools which brought with it diversities in terms of frequencies and types of food consumptions, cultural inert beliefs, socio-economic status, etc. In addition, this approach enabled the youth to have freely expressed themselves through the use of the self-administered questionnaires without any intimidation from the researchers on their personal oral healthcare choices.

However, the sample used during the study represented an approximately 0.84% of the estimated total teenage population for generalization purposes. Another limitation is that a section of teenagers who were out of school during the exercise could not participate in the study. Further, the subjects did not undergo any clinical oral examination to detect their actual dental care status; hence, the reason why results do not make reference to any DMFT/dmft prevalence. Instead as highlighted, data were derived through self-administered questionnaires, a situation which has a likelihood of bringing in confounding variables.

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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  $\mu\text{m}$ , in 2.76  $\mu\text{m}$  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  $\mu\text{m}$ . 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

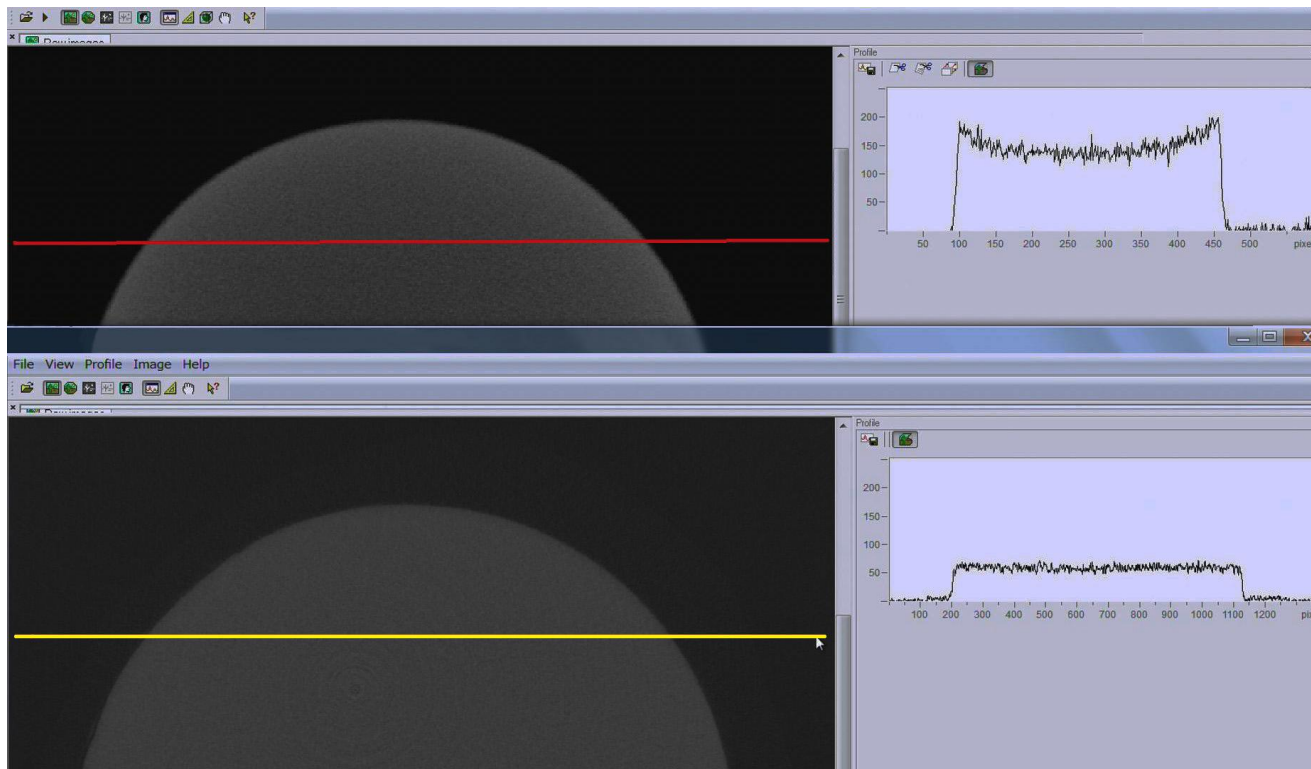
a population-wide level (Marthaler, 1990). Filling damaged teeth with a restoration should not be considered as an ideal treatment since it does not aim at eliminating the fundamental cause of caries (Elderton, 1996). Protective

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  $\beta$ -tricalcium phosphate and sodium lauryl sulfate, to NaF formulations (Karlinsky 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 (Karlinsky et al., 2010a, b, c, 2009a, b, 2011a; Amaechi et al., 2012).

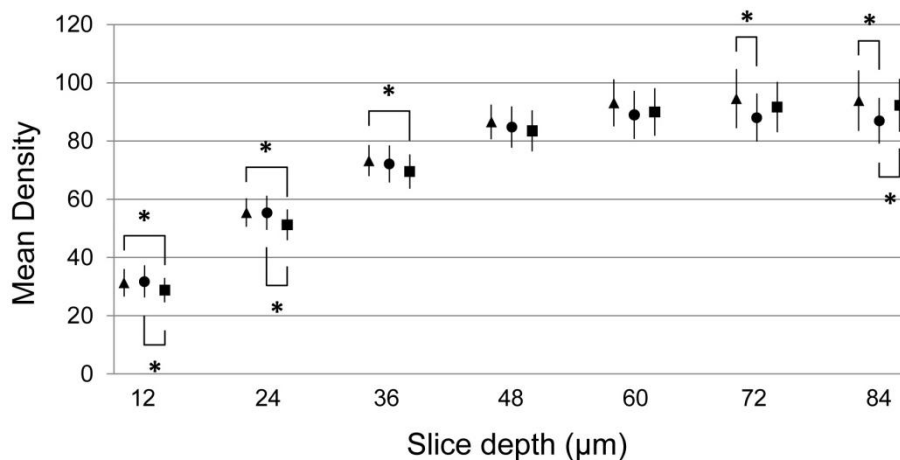
While transverse microradiography, cross-polarization microscopy and microhardness measurements are valuable in the assessment of remineralization/demineralization, another method of assessing efficacy may be through the use of conventional micro computed tomography (micro-CT) which utilizes polychromatic 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- $\mu$ CT, 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- $\mu$ CT, 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<sub>2</sub>O, K<sub>2</sub>HPO<sub>4</sub> (22.07 and 45.83 weight percentage) aqueous solution and SiO<sub>2</sub> (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- $\mu$ CT
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<sub>2</sub>O, K<sub>2</sub>HPO<sub>4</sub> (22.07 and 45.83 weight percentage) aqueous solution and SiO<sub>2</sub> (silica glass).

Parameter	H <sub>2</sub> O	K <sub>2</sub> HPO <sub>4</sub> (22.07 wt%)	K <sub>2</sub> HPO <sub>4</sub> (45.83 wt%)	SiO <sub>2</sub>
<b>LAC at 30 keV</b>	0.38	0.88	1.60	1.91
Density (g/cm <sup>3</sup> )	1.00	1.20	1.46	2.22
<b>LAC at exp</b>	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 ( $\mu$ m) 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 $\times$ 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

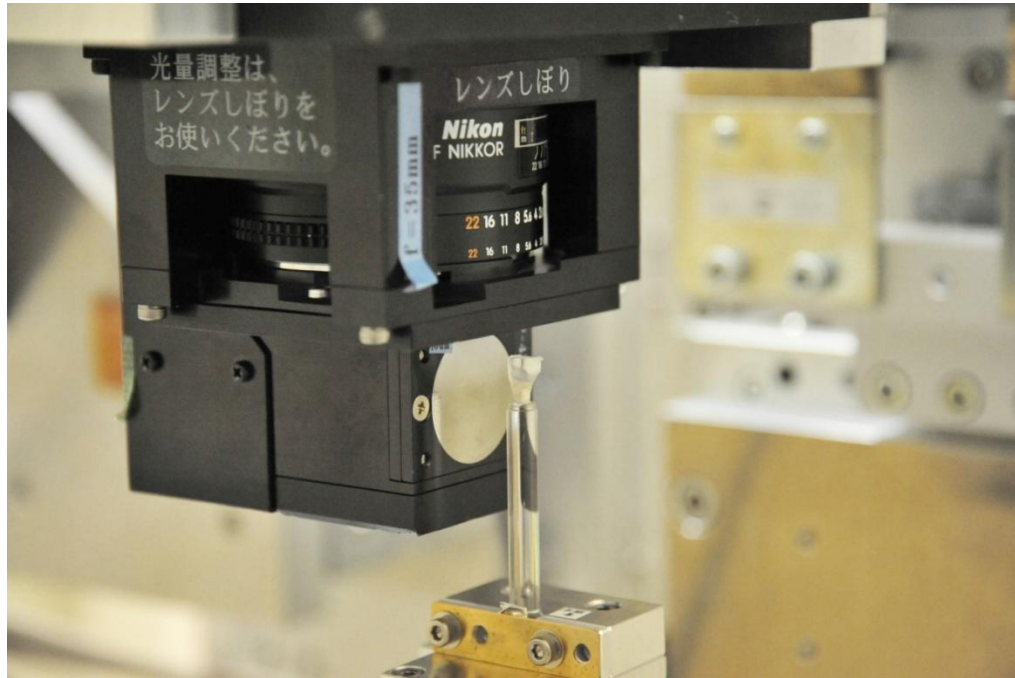
CT values in the present study, or X-ray LAC derived from tomographic reconstructions, are called 'observed

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).

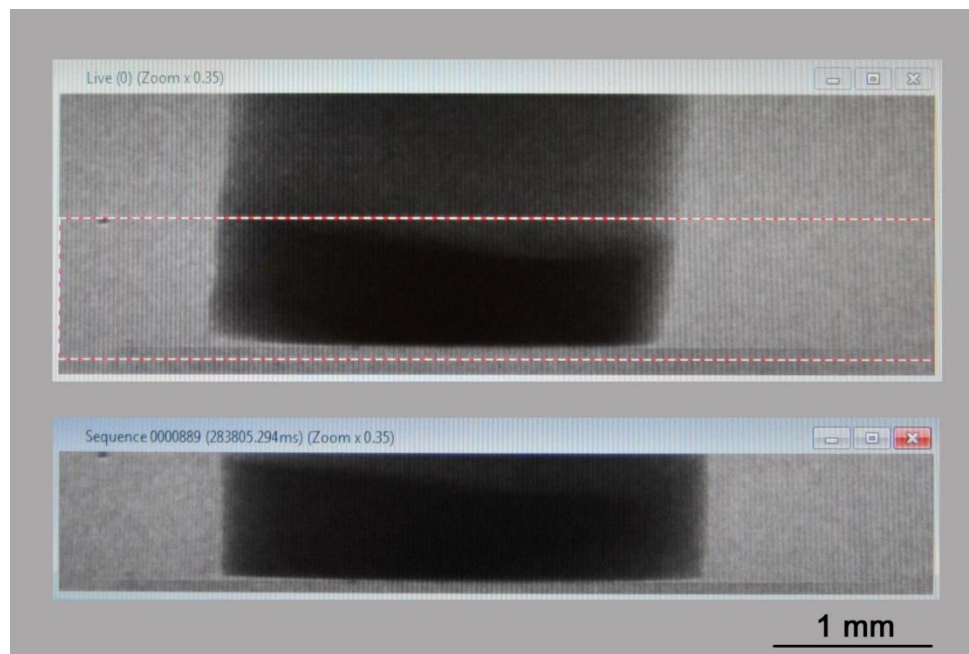
### SR micro-CT

#### *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  $\mu$ m, 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.

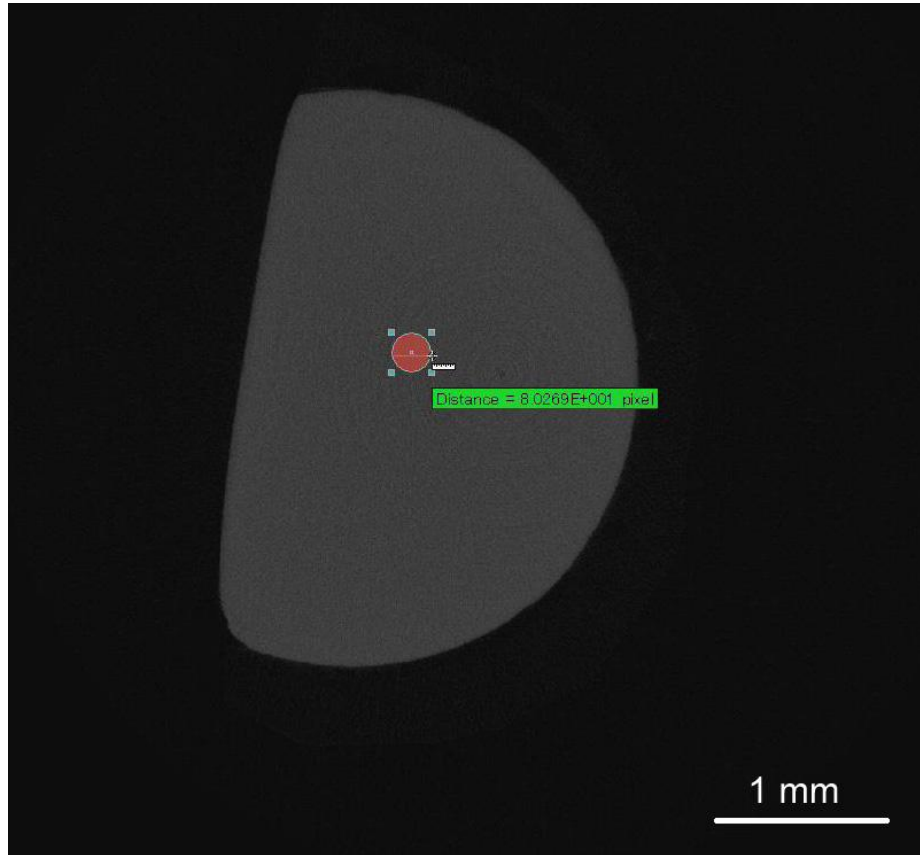


**Figure 4.** Two red dotted lines showing the X-ray FOV in the vertical position. Bovine enamel was set upside down on an acrylic rod using pure water to settle.

### 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.

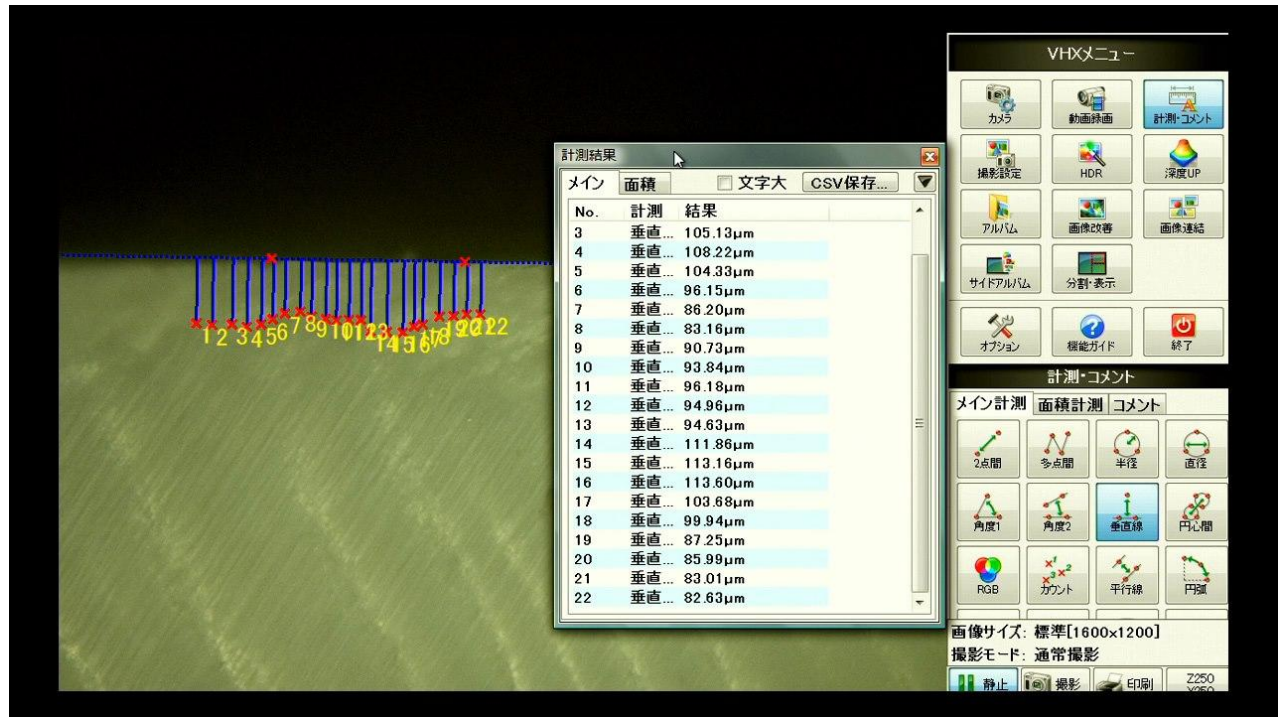


**Figure 5.** An example horizontal view of an enamel specimen. The ROI (red circle) is composed of 6969 pixels. It corresponds to 222  $\mu\text{m}$  in diameter. 4 glass capillaries comprising  $\text{H}_2\text{O}$ ,  $\text{K}_2\text{HPO}_4$  (22.07 and 45.83 weight percent) aqueous solution and  $\text{SiO}_2$  (silica glass) also measured the same ROI size to obtain the X-ray LAC correction curves.

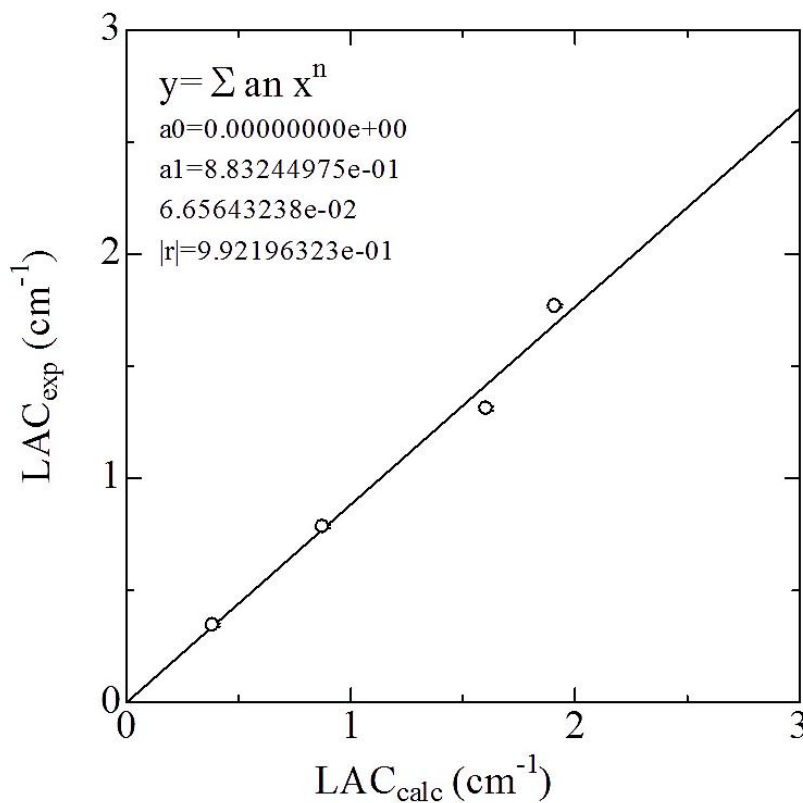


**Figure 6.** An example setup of digital microscopy measurement in cross-sectional view.





**Figure 7.** Lesion depth ( $\mu\text{m}$ ) 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.

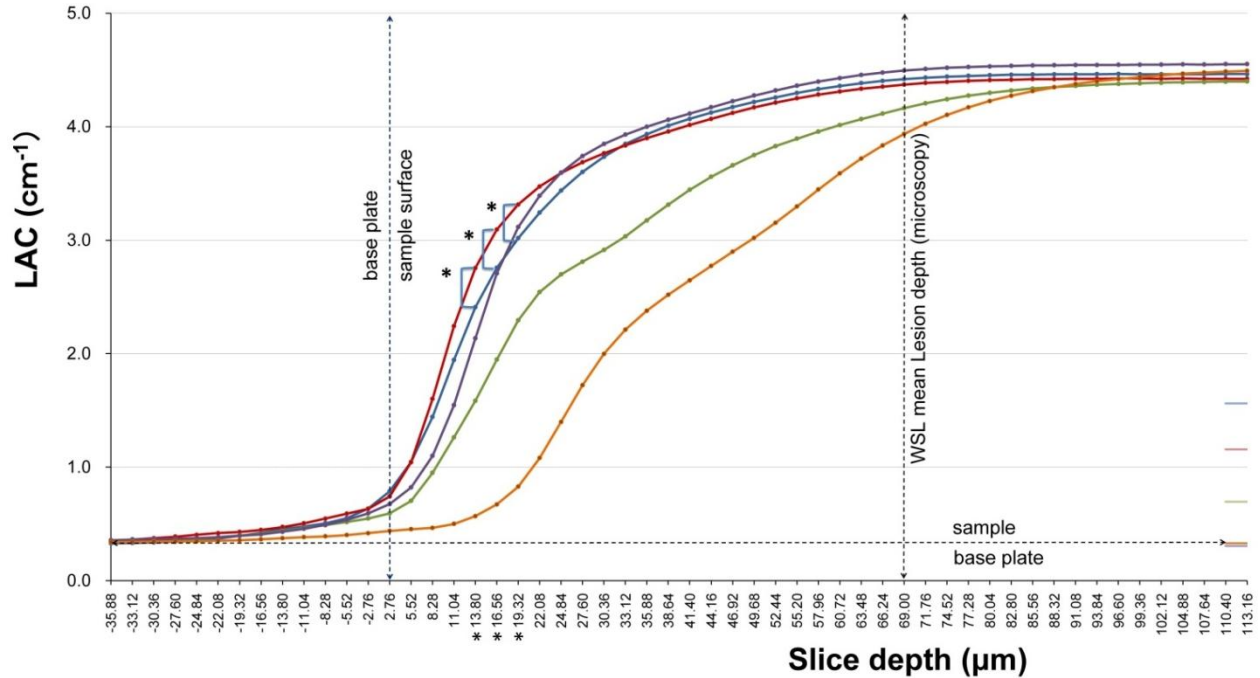
Monochromatic X-rays are used in synchrotron radiation and can eliminate beam hardening effects, which can inadvertently obscure 'true' density measurements of the material under examination. This is important, since the relationship between the CT value and X-ray LAC is not straight forward in almost 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  $\mu\text{m}$ , 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  $\mu\text{m}$ , the SR micro-CT-based densities continue to increase until they plateau around 113.16  $\mu\text{m}$  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.

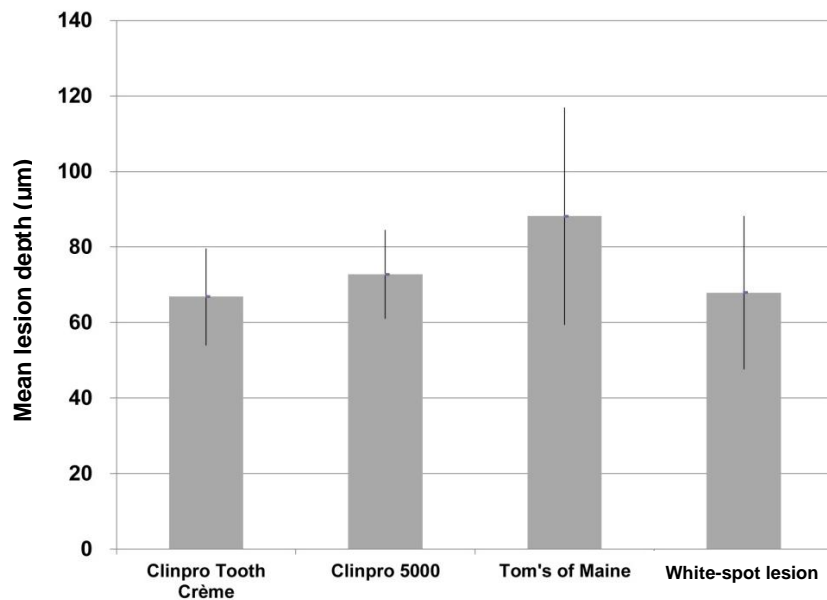
Among the elemental constituents of enamel, calcium is present in the largest concentration (Cate et al., 1988) and is most sensitive to X-ray absorption (Wildenschild et al., 2002). The loss of calcium during initial demineralization of enamel to create the microscopy-based 70  $\mu\text{m}$ -deep

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  $\mu\text{m}$ . 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  $\mu\text{m}$ , 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) (Karlinsky 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 (Karlinsky et al., 2010d; Mensinkai et al., 2012). This view



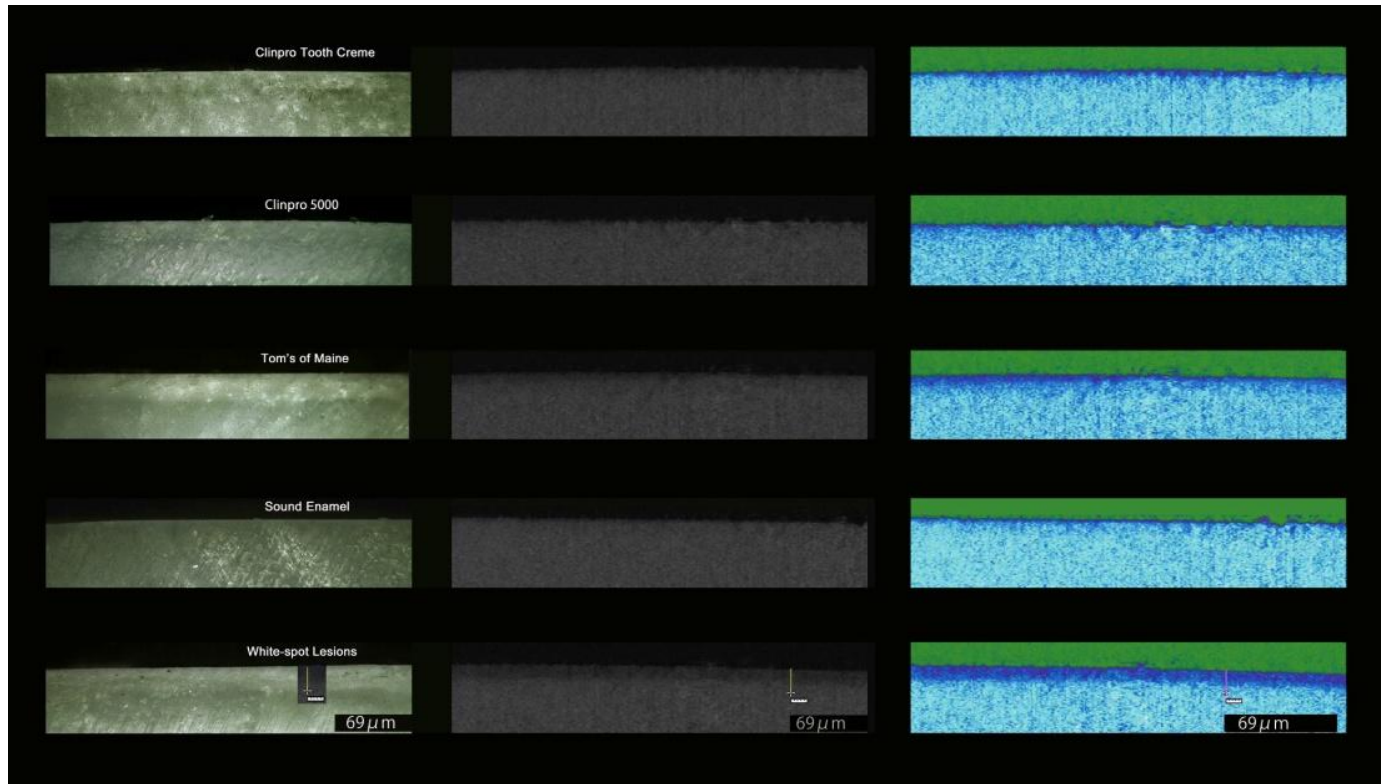
**Figure 9.** X-ray LAC ( $\text{cm}^{-1}$ ) versus slice depth ( $\mu\text{m}$ ) comparisons with Clinpro Tooth Crème (blue) and Clinpro 5000 (red). Images of bovine enamels were obtained with 30 keV X-rays. X-ray LAC ( $\text{cm}^{-1}$ ) versus slice depth ( $\mu\text{m}$ ) for White-spot Lesions treated 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 (Karlinsky and Pfarrer,

2012; Karlinsky 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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The banner features the ODA OREGON DENTAL CONFERENCE logo on the left, which includes the letters "ODA" in a large font and "OREGON DENTAL CONFERENCE" below it. To the right of the logo, the text reads "2014 Oregon Dental Conference®", "April 3-5", "Oregon Convention Center", and "Portland". Further right, the text reads "Dentistry: Where Art Science Meet" with a large, stylized white treble clef graphic.

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### **April 2013**

IDEM Singapore 2014 Scientific Program, April 3 SUNTEC Singapore

### **April 2014**

Oregon Dental Conference April 3-5, 2014 Oregon Convention Center, Portland



# Journal of Dentistry and Oral Hygiene

## Related Journals Published by Academic Journals

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