Cariotester, a new device for assessment of dentin lesion remineralization in vitro

Sachiko UTAKA1, Syozi NAKASHIMA1, Alireza SADR1,2, Masaomi IKEDA3, Toru NIKAIDO1, Akihiko SHIMIZU4 and Junji TAGAMI1,2

1 Cariology and Operative Dentistry, Department of Oral Health Sciences, Graduate School of Medical and Dental Sciences, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Tokyo Medical and Dental University
2 Global COE Program, International Research Center for Molecular Science in Tooth and Bone Diseases, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Tokyo Medical and Dental University
3 Clinical Oral Science, Department of Oral Health Care Sciences, Faculty of Dentistry, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Tokyo Medical and Dental University
4 Department of Dentistry and Oral Surgery, Hyogo College of Medicine, 1-1 Mukogawa-cho Nishinomiya-shi, Hyogo 663-8501, Japan

Corresponding author, Syozi Nakashima; E-mail: s-nakashima.ope@tmd.ac.jp

This study aimed to evaluate the potential of a new device (Cariotester) for monitoring of incipient carious lesion remineralization in root dentin by topical fluoride in vitro. Demineralized bovine dentin specimens were treated by fluoride solutions (APF or neutral NaF) and remineralized for 4 weeks. Cariotester was used to measure penetration depth (CTR depth) of the indenter into the de- and remineralized specimen surface. The specimens were assessed by transverse microradiography (TMR) to determine lesion parameters (depth: LD, mineral loss: ΔZ). Pearson’s correlation analysis showed an overall significant relationship between CTR depth and both TMR parameters. CTR depth appeared to distinguish the positive effect that topical fluoride application had on the remineralization of the outer zone of dentin lesions. Cariotester had the potential to serve as a quantitative tool for monitoring of incipient caries lesion remineralization in root dentin.

Keywords: Cariotester, Dentin remineralization, TMR, Fluoride

INTRODUCTION

It is well known that in industrial countries, elderly population is growing, and the number of teeth retained in this population is also increasing. This growth is accompanied by an increase in the exposed root surfaces1. Therefore, overall, a larger population is at the risk of root caries. On the other hand, hospitalized elderly patients retaining their natural teeth have difficulty to brush their teeth due to physical problems, and root caries a significant oral health concern for them2–4. Given these backgrounds, preventive treatment of the root surface at the caries risk is considered to be an emerging issue in the dental practice today. In this regard, topical fluoride application has been widely accepted as an efficient means of preventing demineralization and boosting remineralization5,6.

In order to evaluate the efficacy of available treatment options involving remineralization rather than surgical intervention, development of a quantitative and objective clinical diagnosis system for root caries assessment is necessary. However, such a modality has not been well established. Assessment of tactile quality of the lesion surface by a dental explorer and visual inspection of the surface (color, texture) are to some extent subjective and not quantitative5,8. Several methodologies using novel instruments have been studied: i.e., Electronic Caries Monitor (Vanguard System, Massachusetts Manufacturing Corporation, Cambridge, MA, USA)9,10, fluorescence measurement DIAGNOdent11 (Kavo, Biberach, Germany) and optical coherence tomography imaging, OCT12. However, a standard clinical system is yet to be introduced.

Recently, a new mechanical device (Cariotester, SUK-971, SaneiME Inc., Yokohama, Japan)13–15 has been registered and introduced in Japanese dental market for clinical hardness assessment of dentin. The principle mechanism of assessment in this device is measuring the hardness based on depth of indenter penetration in situ that can be applied for caries assessment. Fusayama et al.16 reported that in a caries lesion, the infected dentin layer showed the lowest hardness, and that the hardness of the superficial layer could serve as a means of determining the carious activity. In particular, it is claimed that Cariotester is useful to determine dentin hardness and avoid under- and over-excitation during surgical debridement. Featherstone et al.17 and Pugach et al.18 reported that the hardness of enamel and dentin lesions was proportional to their mineral density determined by the gold standard method transverse microradiography (TMR). Therefore, clinical measurement of the hardness of dental surface would be a valid approach for caries assessment, and monitoring of a lesion. However, it is not known whether such a device as Cariotester can actually be used to detect changes in artificial incipient root dentin lesion during remineralization, and the difference among various treatment options.

Acidulated phosphate fluoride (APF) and neutral sodium fluoride (NaF) solutions are most frequently
used by practitioners were examined on dentin lesion remineralization. However, to our knowledge, few studies have been performed on remineralization efficacy of APF and NaF in incipient dentin lesion as professional application19-21).

Therefore, the aim of this study was to evaluate the dentin lesion changes during remineralization after two topical fluoride treatments in artificial incipient root dentin lesions, and to find out if Cariotester was able to quantify these changes as a diagnostic tool. The null hypotheses of the study were that 1- there was no difference in lesion parameters during remineralization of incipient root lesions after application of APF, NaF compared to a control solution. 2- There was no correlation between results obtained by Cariotester and those of TMR in the course of the demineralization and remineralization experiments.

MATERIALS AND METHODS

Specimen preparation
Seventy root dentin blocks, approximately 2×3×3 mm in dimensions, were prepared from freshly extracted sound bovine incisor teeth using a low-speed diamond saw (Isomet 5000, Buehler, Lake Bluff, IL, USA). They were embedded in quick self-curing acrylic resin (UNIFAST II; shade A2, GC, Tokyo, Japan) and the dentin surface were polished using 800 and 1,200 grit silicon carbide abrasive papers (Grit silicon carbide paper, Sankyo Rikagaku, Saitama, Japan). The polished tooth surfaces were covered by acid-resistant nail varnish (MAQuillAGE, RD451, Shiseido, Tokyo, Japan) except for the demineralizing ‘windows’ (1.5×2.5 mm). The covered areas served as the sound dentin area before the demineralization.

Preparation of artificial root dentin lesions and the remineralizing treatment
Figure 1 shows a flow chart of specimen preparation, demineralization, group allocation, and assessments by Cariotester and TMR in this study. The polished dentin specimens were demineralized for 1 week in demineralizing solution (1.5 mM CaCl2, 0.9 mM KH2PO4, 50 mM acetic acid, 3.08 mM NaN3, pH 4.7 adjusted by NaOH) at 37°C. The demineralized specimens were allocated to 4 groups as follows; Baseline group: 10 blocks were used in this untreated group without remineralization that served as a baseline control; No-fluoride group: 20 blocks served in the group for remineralization without fluoride treatment; APF group: 20 blocks were treated by APF, prepared by mixing 2% sodium fluoride in 0.1 M phosphoric acid in deionized water; NaF group: the remaining 20 blocks were treated by 2% neutral sodium fluoride solution followed by remineralization.

APF and NaF solutions both contained 9,048 ppm F. A drop (3 µL) of each fluoride solution was applied only on the ‘window’ area of demineralized dentin surface for 30 min, according to the standard application procedure followed by brief rinsing with deionized water. The specimens in the No-fluoride, APF and NaF groups were then separately immersed in 33 mL of a remineralizing solution (1.0 mM CaCl2, 3.0 mM KH2PO4, 100 mM NaCl, 3.08 mM NaN3, pH 6.5 adjusted by NaOH) for either 2 or 4 weeks at 37°C (n=10). The concentrations of CaCl2 and KH2PO4 in the remineralizing solution was referred to a previous study22), and the solution was refreshed twice a week.

Measurement of Cariotester indentation depth (CTR depth)
The indentation depth of de- and remineralized specimens, now on abbreviated as CTR depth, was assessed using the Cariotester system, as described in Fig. 2. The system is composed of a handpiece equipped with an indenter, a digital microscope and a notebook computer. The conical angle of the tungsten carbide indenter is 50 degrees, and its apex is rounded with a radius of 18-μm. A white water-based poster color is applied to the apical portion of the indenter before each indentation, and the indentation is manually performed by pushing the handpiece against the tooth surface, to reach a load of 150 g in 1 s. A pressure sensor alerts when the force reaches the 150 g target. After the indentation, the apical portion of the indenter is observed under the digital microscope, and CTR depth (μm) is registered. CTR depth is obtained by measuring the distance between the apex of the indenter and the border of the remaining poster color using the Cariotester software installed on the notebook computer.

For each specimen, 3 indentations were performed at three randomly selected locations and a mean value of the 3 CTR depths was used as the representative data of that specimen. CTR depth measurement was done by one of the present authors SU throughout this study.
**Fig. 2** The Cariotester comprises two main components. (a) a digital microscope attached to a personal computer to measure the depth of indentation; (b) a handpiece equipped with the indenter. The CTR depth is the distance between the apex and horizontal line (L) of the remaining poster color. (c) schematic presentation of Cariotester indenter in contact with the lesion surface. Note that depending on the lesion depth and structure, the indenter may not penetrate up to the lesion front under the applied force.

**Fig. 3** (a) Representative TMR digital image of a baseline specimen in this study; (b) TMR depth vs. mineral density profile with representation of various parameters compared against CTR depth in this study; LD: lesion depth; \( \Delta Z \): integrated mineral loss; \( \Delta Z_{\text{CTR}} \): mineral loss up to the CTR depth from Cariotester.

**TMR analysis**
De- and remineralized specimens were cut into thin sections with approximately 200–230 \( \mu \)m in thickness using the Isomet saw for TMR. The sections were kept in 70% glycerin solution to minimize the lesion shrinkage until the x-ray exposure TMR images were taken using an x-ray generator (CMR-2, SOFTEX, Tokyo, Japan) on the x-ray glass plate film (High Precision Photo Plate PXHW, Konica Minolta Photo Inc., Tokyo, Japan) under the conditions of 20 kV, 3.0 mA for 6 min with Ni filter, together with aluminum step-wedge. The images were stored as the digital images (gray values from 0–256) through scanning of the developed TMR films using a CCD camera (Olympus DP70, Olympus Corp., Tokyo, Japan) and a microscope (Olympus BX41, Olympus Corp., Tokyo, Japan). By use of a customized image processing software (ImageJ; version1.42q, Wayne Rasband, NIH, USA), mean depth-profiles of the lesion images over approximately 500 \( \mu \)m of the lesion window were created, and the lesion depth (LD: \( \mu \)m) and mineral loss (\( \Delta Z \); vol% \( \mu \)m) were obtained\(^\text{12} \)). Furthermore in this study, \( \Delta Z_{\text{CTR}} \) (vol% \( \mu \)m) was introduced and measured as the integrated mineral loss from the lesion surface to the indentation depth. Figure 3 shows the details of each parameter calculation from the TMR mineral density profiles.

**Statistical analysis**
The data of TMR lesion parameters (LD, \( \Delta Z \), \( \Delta Z_{\text{CTR}} \)) and CTR depth at the baseline and after remineralization for 2 and 4 weeks in each group were analyzed by the Wilcoxon rank sum test with Bonferroni correction,
which was the non-parametric test of choice for the non-normal data distribution in this study. The data were compared within each treatment group by time (Baseline, 2 or 4 weeks), and between the treatment groups at each remineralization period. Moreover, Pearson's correlation coefficient (r) and confidential interval (CI) were calculated to elucidate relationship between Cariotester (CTR depth) and TMR (LD, ΔZ or ΔZ\textsubscript{CTR}), within each treatment group separately as well as for the overall average data; in this manner 7 averaged data points (6 from the 3 treatment groups at week 2 or 4 in addition to one from baseline) were employed. These analyses were performed with a statistical software (SPSS; version 16; SPSS ver16; Chicago, IL, USA) at significance level of a=0.05.

**RESULTS**

A representative TMR image is shown in Fig. 3. In any group, TMR images showed cavitated lesion without formation of a surface layer even after the remineralization. Figure 4 shows longitudinal changes in LD, ΔZ and ΔZ\textsubscript{CTR} for each of the 3 treatment groups.

Table 1 exhibits pair comparisons between experimental periods (Baseline, 2 or 4 weeks remineralization). The statistical analysis showed significant reductions in both LD and ΔZ at week 4 for No-fluoride group, and at week 2 and 4 for the fluoride groups compared to the baseline (p<0.05). These results indicated that all groups showed lesion remineralization. However, no statistical difference was noted between week 2 and week 4 within any of the groups (p>0.05).

Statistical analysis between groups showed that for LD, there was no significant difference among the three groups at both week 2 and 4 (p>0.05). Whereas for ΔZ, a significant difference was only found between No-fluoride and NaF at week 2 (p<0.05), while there was no statistical difference among the 3 groups at week 4 (p>0.05).

Statistical analysis of CTR depth showed significant difference between control and fluoride groups at both week 2 and week 4 (p<0.05). However, no difference in CTR depth among the 2 fluoride groups was noted at any time (p>0.05). The results of ΔZ\textsubscript{CTR} closely followed a similar trend as that of CTR depth.

Table 2 shows Pearson’s correlation coefficients and their significance between the CTR depth and TMR lesion parameters (LD, ΔZ, ΔZ\textsubscript{CTR}) for individual groups. Except for the case of LD in control group, statistically significant correlations were found (p<0.05). Generally, higher r values were noted in fluoride groups. Furthermore, the overall correlations between Cariotester and TMR parameters, obtained by plotting average CTR depth from each study group against corresponding average LD, ΔZ or ΔZ\textsubscript{CTR}, are presented in Fig. 5. Higher correlations were noted (r=0.86, p<0.05 for LD, r=0.80, p<0.05 for ΔZ and r=0.92, p<0.005 for ΔZ\textsubscript{CTR}).

Figure 6 shows TMR profiles of the Baseline and all remineralization groups at 4 weeks. The profiles

---

**Fig. 4** Longitudinal changes in various study parameters.

(a) LD; (b) ΔZ; (c) CTR depth and (d) ΔZ\textsubscript{CTR}. Among TMR parameters, ΔZ\textsubscript{CTR} shows the close trend to that of CTR depth.
Table 1  Statistical analyses of parameters measured by TMR or Cariotester at different remineralization periods

LD (mean SD)

<table>
<thead>
<tr>
<th></th>
<th>No-fluoride</th>
<th>APF</th>
<th>NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 w (Baseline)</td>
<td>403 (51)Aa</td>
<td>311 (27)Ch</td>
<td>343 (32)Dc</td>
</tr>
<tr>
<td>2 w</td>
<td>359 (62)Abc</td>
<td>311 (27)Ch</td>
<td>343 (32)Dc</td>
</tr>
<tr>
<td>4 w</td>
<td>332 (35)Bd</td>
<td>324 (21)Cd</td>
<td>335 (23)Dd</td>
</tr>
</tbody>
</table>

ΔZ (mean SD)

<table>
<thead>
<tr>
<th></th>
<th>No-fluoride</th>
<th>APF</th>
<th>NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 w (Baseline)</td>
<td>9,291 (1151)Aa</td>
<td>8,027 (603)Bbc</td>
<td>7,374 (1059)Cc</td>
</tr>
<tr>
<td>2 w</td>
<td>8,608 (710)Ab</td>
<td>8,027 (603)Bbc</td>
<td>7,374 (1059)Cc</td>
</tr>
<tr>
<td>4 w</td>
<td>7,300 (1057)d</td>
<td>7,474 (1144)Bd</td>
<td>7,113 (1422)Cd</td>
</tr>
</tbody>
</table>

CTR depth (mean SD)

<table>
<thead>
<tr>
<th></th>
<th>No-fluoride</th>
<th>APF</th>
<th>NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 w (Baseline)</td>
<td>218 (10)a</td>
<td>160 (12)Bh</td>
<td>163 (10)Ch</td>
</tr>
<tr>
<td>2 w</td>
<td>196 (17)A</td>
<td>160 (12)Bh</td>
<td>163 (10)Ch</td>
</tr>
<tr>
<td>4 w</td>
<td>188 (7)A</td>
<td>160 (8)Bc</td>
<td>158 (9)Cc</td>
</tr>
</tbody>
</table>

ΔZ_{CTR} (mean SD)

<table>
<thead>
<tr>
<th></th>
<th>No-fluoride</th>
<th>APF</th>
<th>NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 w (Baseline)</td>
<td>7,129 (390)Ab</td>
<td>5,339 (658)Bb</td>
<td>4,851 (1098)Cb</td>
</tr>
<tr>
<td>2 w</td>
<td>7,415 (471)A</td>
<td>5,339 (658)Bb</td>
<td>4,851 (1098)Cb</td>
</tr>
<tr>
<td>4 w</td>
<td>6,112 (1042)</td>
<td>4,700 (594)Bc</td>
<td>4,224 (710)Cc</td>
</tr>
</tbody>
</table>

In each column, values marked by the same superscript uppercase letters are not significantly different (p>0.05).
In each row, values marked by the same superscript lowercase letters are not significantly different (p>0.05).

Table 2  Pearson’s correlation between CTR depth and TMR parameters in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>TMR parameters</th>
<th>r</th>
<th>p</th>
<th>95% CI of r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD</td>
<td>0.320</td>
<td>0.085</td>
<td>-0.046–0.610</td>
</tr>
<tr>
<td>No-fluoride</td>
<td>ΔZ</td>
<td>0.417</td>
<td>&lt;0.05</td>
<td>0.067–0.676</td>
</tr>
<tr>
<td></td>
<td>ΔZ_{CTR}</td>
<td>0.378</td>
<td>&lt;0.05</td>
<td>0.021–0.650</td>
</tr>
<tr>
<td></td>
<td>LD</td>
<td>0.684</td>
<td>&lt;0.001</td>
<td>0.430–0.838</td>
</tr>
<tr>
<td>APF</td>
<td>ΔZ</td>
<td>0.496</td>
<td>&lt;0.01</td>
<td>0.166–0.727</td>
</tr>
<tr>
<td></td>
<td>ΔZ_{CTR}</td>
<td>0.872</td>
<td>&lt;0.001</td>
<td>0.747–0.938</td>
</tr>
<tr>
<td></td>
<td>LD</td>
<td>0.531</td>
<td>&lt;0.005</td>
<td>0.211–0.748</td>
</tr>
<tr>
<td>NaF</td>
<td>ΔZ</td>
<td>0.489</td>
<td>&lt;0.01</td>
<td>0.157–0.722</td>
</tr>
<tr>
<td></td>
<td>ΔZ_{CTR}</td>
<td>0.827</td>
<td>&lt;0.001</td>
<td>0.664–0.914</td>
</tr>
</tbody>
</table>

were created by plotting average data of 10 specimens for each group. Note that fluoride containing groups resulted in higher mineral density at the outer lesion zone (<200 μm), while the no-fluoride group showed better inner zone remineralization.

DISCUSSION

TMR is considered as the gold standard method for determination of mineral density and depth of carious enamel and dentin lesions in vitro. Given that there is no gold standard method accepted for clinical monitoring of root caries, for validation of a newly developed device such as Cariotester against the gold standard, an in vitro experiment would be essential. Nevertheless, clinical application of a device may be totally different from the in vitro experiment conditions. For instance, in the current study, the measurements were performed on polished flat dentin surfaces, and the CTR depth values appeared to be reproducible with small relatively standard deviations. However, in the actual clinical situation, the dental surfaces vary in terms of both orientation and roughness, depending on factors such as anatomical features. It is known that these topographic characteristics affect indentation hardness measurements. Therefore, assessment of Cariotester performance for natural lesions and under clinical situation requires future study.

Results of TMR demonstrated longitudinal lesion remineralization in all study groups. The remineralization trend was reflected in CTR depth results, which clearly indicated that a reduction in CTR depth could be regarded as the consequence of an effective remineralization process; however, the individual correlation coefficient values between CTR depth and the standard TMR parameters were not very high. It should be noted that CTR depth is a parameter that depends on the balance between the downward pressure load and the upward resistance from the underlying lesion structure that consists of mineral and collagen at various densities. In order to limit the physical damage induced by the indentation process, Cariotester applies a fixed load of 150 g; therefore, in a deep lesion, a remarkably large unreachable depth beneath the indenter may remain. Such case particularly explains the lack of correlation between CTR depth and LD at the No-fluoride group (Table 2).

On the other hand, there were significant differences in CTR depth values between No-fluoride and fluoride groups after 4 week remineralization. In order to explain this finding, a closer look at the lesion structure would be necessary. As seen in Fig. 6, both fluoride containing groups resulted in a remarkable increase in the mineral density of the approximately first 200 μm below surface (outer zone), while in the No-fluoride group, remineralization dominantly occurred at deeper layers. In this study, the ΔZ_{CTR} parameter was introduced as the integrated mineral loss down to just the CTR depth to evaluate the validity of Cariotester results when concerning the shallower subsurface structure. Interestingly, this new parameter confirmed...
the difference found by Cariotester between No-fluoride and fluoride groups at 4 weeks while the difference was not found by ΔZ and LD.

Several lines of literature have emphasized on the effect of high concentration topical fluoride application on lesion remineralization. It appears that the application of the high concentration fluoride solutions resulted in formation of a CaF₂-like material, leading to a more pronounced remineralization at the outer zone for both APF and NaF groups, explaining CTR depth and ΔZCTR results which were in turn confirmed by TMR profiles (Fig. 6).

In general, given that the overall correlations between CTR depth and LD or ΔZ were acceptable (Fig. 5), the results of Cariotester appeared to be valid in terms of root dentin lesion assessment. In particular, stronger correlation between CTR and ΔZCTR suggested that Cariotester approach was valid for assessment of outer zone of the lesion. Further study is warranted to confirm the accuracy of dentin lesion assessment by Cariotester under various types of lesions with different structures, i.e., shallower lesions with and without surface layer. Most importantly, clinical trials are necessary to approve the utility of this device as a chair-side tool.

In conclusion, this in vitro study demonstrated the potential of Cariotester as a quantitative diagnostic tool for monitoring of incipient carious lesion remineralization in root dentin.

ACKNOWLEDGMENTS

This work was supported by a grant for Global COE Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University.

REFERENCES