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Title: Objective and Quantitative Assessment of Caries Lesion Activity

Short Title:

Objective Caries Lesion Activity Assessment

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Abstract

Objectives: Evaluate the ability of objectively measured specular reflection, roughness, and fluorescence change during dehydration to assess caries lesion activity. **Methods:** One hundred ninety-five ground/polished 3x3x2mm sound human enamel specimens were divided into three groups and demineralized using a multispecies microbial caries model for 3, 6, or 9 days; and then remineralized with 1,100 ppm-F as NaF solution for 10 days using a pH-cyclic model. Reflection (amplitude: %), roughness (Ra: μm), fluorescence change during dehydration (ΔQ: %×mm^2), and microfocus computed tomography [μ-CT: lesion volume (μm^3)] were measured for sound, demineralized and remineralized enamel. The surface was hydrated and fluorescence images were acquired at 1s intervals for 10s (ΔQ_{10}). During image acquisition, surface was dehydrated with continuous compressed air. Changes-in-ΔQ per second (ΔQ_D: %×mm^2/sec) at 5 (ΔQ_{D5}) and 10s (ΔQ_{D10}) were obtained. **Results:** Reflection decreased from sound to

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demineralized groups (p<0.0001); remineralized groups were higher than demineralized groups (p<0.001), but not different from sound (p>0.32). Roughness increased from sound to demineralized groups (p<0.0001) and remineralized groups were also higher than sound (p<0.0001). ΔQ_{10}, ΔQ_{D5} and ΔQ_{D10} increased from sound to demineralized groups (p<0.0001), and remineralized groups decreased compared to demineralized groups (p<0.05), but was higher than sound (p<0.0001). The correlations of µ-CT with reflection, roughness, and ΔQ_{10} were -0.63, 0.71, and 0.82, respectively (p<0.0001). **Conclusions:** Reflection, roughness and ΔQ could distinguish between sound and demineralized enamel. Reflection and ΔQ were able to distinguish between demineralized and remineralized enamel. **Clinical significance:** Determination of caries activity, whether a lesion is active or inactive, is an essential and critical component of caries diagnosis. However, especially for enamel lesions, it is difficult to estimate without longitudinal follow-up. Reflection, roughness and fluorescence change during dehydration have the potential to measure caries lesion activity at a-single-appointment.

Keywords: Caries lesion activity; reflection; roughness; fluorescence imaging; demineralization; remineralization.

Introduction

Although the prevalence of dental caries has decreased, the disease is prevalent in all age groups [1]. A systematic review and metaregression showed that in 2010, untreated caries in permanent teeth was the most prevalent condition worldwide, and untreated caries in deciduous teeth was the 10th most prevalent condition [2]. A lesion considered to be progressive, where the lesion continues to demineralize, would be described as an active caries lesion. A lesion that has stopped further progression (stagnant/remineralized) is referred to as an inactive/arrested caries lesion [3]. Determination of caries lesion activity, whether a caries lesion is active or inactive/arrested caries lesion, is an essential and critical component of appropriate caries diagnosis. Therefore, activity assessment is of paramount importance for correct clinical decision-making for management of caries lesions at the time of examination and treatment planning process. However, the activity of non-cavitated enamel lesions (white-spot lesions) is difficult to estimate with currently available methods without longitudinal examinations. Previous studies demonstrated objective evaluation of caries lesion activity [4, 5, 6]. An infrared camera was used to measure the temperature descent at the demineralized enamel surface during dehydration [4]. The results indicated that positive correlations were found between the parameters of the infrared

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camera and those of the gold standard. Pulse thermography was used to measure the thermal response of sound, demineralized and remineralized enamel [5]. By measuring the spatial thermal decay time, this method has the potential to quantify differences among these three stages of enamel surface status. Near-infrared reflectance imaging was used to measure the degree of enamel remineralization and determine its correlation with the rate of water loss [6]. It appeared that near-infrared imaging is suitable to detect enamel remineralization during dehydration. Despite the success of these studies, one of the limitations was their cross sectional design. None of the studies demonstrated the ability of these methods to follow longitudinally sound, demineralized and remineralized stages.

The fundamental hypothesis of this current study is that enamel caries lesion activity is measurable and it can be determined by monitoring changes in the degree of tooth surface porosity. This hypothesis is based on several observations. At the surface level, the initial stage of demineralization is characterized by development of microchannels and surface softening [7 – 10]. As demineralization progresses over time, these microchannels become wider and longer [11]. When enamel surfaces are hydrated/wet and in an inactive stage, these surfaces should be smoother than surfaces that have microchannels. In addition, when light rays strike a smooth surface, the reflected rays are parallel to each other. This is known as specular reflection and surfaces that cause specular reflection appear shiny. Dehydrated/dried surfaces and those presenting microchannels result in an irregular, rough surface that reflects light rays in various directions. This causes the surface to appear dull (non-glossy) as seen in active caries lesions. Ando et al., [12] demonstrated as the surfaces demineralized, reflection (appearance) measured by optical reflectometry was decreased and roughness (texture) measured by surface profilometry was increased. These results suggest the possibility of characterizing enamel demineralization objectively and quantitatively by measuring specular reflection and roughness.

When non-cavitated enamel lesions are hydrated/wet, microchannels are filled with water and the amount of water is larger in active lesions (demineralized enamel) than in inactive lesions (remineralized enamel). The difference between the refractive index of water (1.33) and that of enamel crystal (1.62) is minimal, and the air has a lower refractive index (nearly 1.0). When the non-cavitated lesion, in either an active or inactive stage, is dehydrated with air filling porous areas, the amount of light scattering increases. As the surface dehydrates, the lights scattering is more pronounced in demineralized enamel (active stage) than in remineralized enamel (inactive stage). Thus, the hypothesis of this study was that the rate of vaporization of water in the lesion body during dehydration, as measured by fluorescence change determined by quantitative light-induced fluorescence (QLF) technique, would indicate caries lesion activity (Fig. 1). The rate of

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evaporation (vaporization) would be more pronounced in active lesions than in inactive lesions; therefore, the change-in-QLF variables per second (ΔQLF_D) during dehydration would be distinctly different between active and inactive lesions. Studies have demonstrated the possibility of the QLF technique to assess caries porosity as a surrogate of caries lesion activity [13, 14]. Ando et al., [15] demonstrated in extracted human teeth that during the first few seconds of dehydration by continuous-compressed air, the change-in-QLF variables per second (ΔQLF_D) for active lesions was larger than those of inactive lesions. This suggests that ΔQLF_D during the first few seconds of dehydration by continuous compressed air might be able to differentiate between active (demineralizing) and inactive (remineralized) caries lesions at the time of examination. Pilot clinical study also revealed that QLF variables, such as lesion size and ΔQ, during dehydration indicated increments for lesions designated as active and minimal change for lesions defined as inactive [16].

These previous studies demonstrated that reflection, roughness and QLF variables during dehydration could monitor/follow progression of demineralized enamel. Moving towards the next step, it is important to demonstrate these techniques could monitor regression of demineralized enamel (remineralization). Therefore, the primary objective of this study was to evaluate whether enamel caries lesion activity could be determined objectively by specular reflection and roughness. The secondary objective was to evaluate the validity and reliability of objective and quantitative means of real-time fluorescence imaging technology for assessing caries lesion activity. Our hypotheses were: the active lesion stage (demineralization) would present less reflection and greater roughness values than the inactive stage (remineralization); and the change-in-QLF variables per second for the active lesion stage would present larger values than that for the inactive lesion stage.

Materials and Methods

Specimen Preparation

Extracted human teeth were collected from dental practitioners in the State of Indiana and transported in 0.1% thymol solution. The collection of human teeth for use in dental laboratory research studies has been approved by the Indiana University (IU) Institutional Review Board (IRB). Sound permanent incisors were sterilized with ethylene oxide gas. From these teeth, 195 3×3×2 mm blocks were prepared. The exposed specimens' enamel surface was polished using a 1-µm diamond polishing suspension on a polishing cloth by RotoForce-4 and RotoPol-31 (Struers Inc., Rødovre, Denmark).

Multiple Species Microbial Demineralization

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These enamel specimens were randomly divided into three groups (n=65/group) and demineralized using a multispecies microbial caries model [*Actinomyces naeslundii, Lactobacillus casei, Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguinis*] for 3 (Demin 3d), 6 (Demin 6d), or 9 days (Demin 9d) [17, 18]. Culture medium was supplied with 0.75 ppm-F solution (as NaF) and with 3% sucrose.

pH-Cycling Remineralization

Specimens were then remineralized with 1,100 ppm-F solution (as NaF) using a pH-cyclic model [19] for 10 days: Demin 3d-remin, Demin 6d-remin, or Demin 9d-remin. The collection of human saliva for use in dental laboratory research studies has been approved by the IU IRB.

Overall Study Procedure

Measurements for sound, demineralized and remineralized enamel were obtained by reflectometry (reflection), profilometry (roughness), quantitative light-induced fluorescence (fluorescence change), and microfocus computed tomography (mineral content).

Reflectometry

Specimens were placed inside the slot of a custom-made specimen holder. Reflectivity (reflection) was measured using a computer-guided optical spectrometer configured as a reflectometer (AvaSoft version 7.1.0 Full, Avantes Inc, Broomfield, CO, USA). The system was calibrated using natural human sound enamel as reference material prior to analysis. A fiber optic reflection probe (FCR-7UV100-2-1.5×100, Avantes Inc, Broomfield, CO, USA) was placed perpendicular to the specimen surface using a special angled fiber holder (AFH-15, Avantes Inc, Broomfield, CO, USA). Average reflectance (reflection) [amplitude, %] was determined for each specimen.

Profilometry

Roughness measurements were determined by computer-guided light profilometry (Proscan 2000A, Scantron Industrial Products Ltd., Taunton, UK). The Proscan features high speed measurement up to 2,000 points/second coupled with a high resolution of 0.003 µm. The profilometric data was digitally recorded and processed by dedicated software (Proscan 2000A, Scantron Industrial Products Ltd., Taunton, UK). In this study, the parameters were limited to arithmetical mean roughness; Ra. Ra is the most commonly used surface roughness parameter.

Light-induced Fluorescence

Fluorescence change was obtained by Quantitative Light-induced Fluorescence technique (QLF 2.00g, Inspektor Research Systems B.V., Amsterdam, The Netherlands). The surface was hydrated with a wet cotton pellet by deionized water for 60 seconds and a series of fluorescence images was acquired at 0, 5, and 10 seconds after dehydration. During image acquisition, specimens were dehydrated with continuous compressed air. Dehydration/image acquisition time

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was determined based on previous studies [15, 16]. Among the QLF variables [fluorescence loss (ΔF: %), area (mm^2) and ΔQ (%×mm^2)], ΔQ was chosen for analysis based on previous studies [16, 20]. ΔQ (%×mm^2) at 10 seconds (ΔQ_{10}: %×mm^2) and changes-in-ΔQ per second (ΔQ_D: %×mm^2/sec) at 5 (ΔQ_{D5}) and 10 seconds (ΔQ_{D10}) were obtained in the manner described by Ando et al. [15].

Microfocus Computed Tomography

The microfocus computed tomography (μ-CT) images of five specimens from each group (total of 15 specimens) were acquired using SkyScan 1172 (Aartselaer, Belgium). The scanning procedure was performed using 65kV, 153 μA, and 2.25 μm pixel size. Lesion volume (μm^3) was determined using gray scales between 87% and 30% of the sound enamel in the manner described by Zhang et al. [21].

Statistical Analyses

The lesion stages were compared using a mixed-model ANOVA with a random effect for specimen to account for the non-independence of the repeated measurements made on a specimen, allowed for different variances for each lesion stage, and allowed correlations among lesion stages to vary. A multiple comparisons adjustment was applied to control the overall significance level 5%. The analyses for all measurements were performed using the ranks of the data.

Sample Size Calculation

Sample size calculations were performed prior to study onset. All sample size calculations were performed using PASS 2005 (NCSS, Kayesville, UT). For simplicity, sample size calculations for within-specimen comparisons among lesion stages were performed using paired t-test calculations assuming a within-specimen correlation of 0.2. Between-specimen comparisons among the demineralization groups were performed using two-sample t-test calculations. For comparisons among the lesion stages at an overall 5% significance level, a sample size of 50 specimens per demineralization group would provide 90% power to detect effect sizes of 0.577 within each demineralization group and 0.325 for all demineralization groups combined between any two lesion stages.

For comparisons among all demineralized and remineralized groups at an overall 5% significance level, the sample size would provide 90% power to detect an effect size of approximately 1.0 between any two groups. Standard deviation estimates from a previous study of ΔQ_D at 5 seconds ranged from 9-16 relative units per second for various demineralized and remineralized groups, with mean changes ranging from 25-118 relative units per second. The previous study used chemical demineralization, so we assumed a higher standard deviation of 30 relative units per

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second. Therefore, effect sizes of 1.0, 0.577, and 0.325 would represent differences of 30, 18, and 10 relative units per second. For reflection and roughness, standard deviation estimates based on a pilot study were 30% and 0.60 μm, respectively. Therefore, effect sizes of 1.0, 0.577, and 0.325 represented differences of 30%, 18%, and 10% for reflection and 0.60 μm, 0.35 μm, and 0.20 μm for roughness.

Results

Table 1 presents the average and standard error of each method.

Reflectometry

Reflection (amplitude) in all demineralized groups was statistically lower than sound ($p<0.0001$). After remineralization, reflection was higher for all remineralized groups compared to demineralized groups ($p<0.001$), but there were no significant differences among sound and remineralized groups ($p>0.32$).

Profilometry

The roughness (Ra) of all demineralized groups was statistically larger than that of sound ($p<0.0001$). Roughness of remineralized enamel after 6-days of demineralization (Demin 6d-remin) was larger than that of 6-days demineralized enamel (Demin 6d: $p=0.0393$), but no other comparisons between demineralized and remineralized groups were significant ($p>0.28$). However, all remineralized groups were significantly larger than sound ($p<0.0001$).

Light-induced Fluorescence: Severity Assessment

ΔQ_{10} on all demineralized groups was statistically higher than sound ($p<0.0001$); however, among the demineralization groups, there was a significant difference between groups only between 3-days of demineralization (Demin 3d) and 9-days of demineralization (Demin 9d) ($p=0.0010$). After remineralization, ΔQ_{10} became significantly lower compared to demineralized groups ($p<0.02$) with the exception of 9-days demineralization followed by 10-days remineralization (Demin 9d-remin) not significantly different from Demin 3d ($p=0.18$). Conversely, ΔQ_{10} of all remineralized groups were higher than that of sound enamel ($p<0.0001$). Additionally, within the remineralization groups, ΔQ_{10} was higher with increased demineralization days ($p<0.0001$).

Light-induced Fluorescence: Activity Assessment

Figure 2 presents the averages and standard errors of ΔQ_{D5} and ΔQ_{D10}. For both dehydration times, after demineralization, ΔQ_D was increased for all demineralization groups ($p<0.0001$), but was only significantly different between Demin 3d and Demin 9d ($p<0.05$). After remineralization,

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ΔQ_D was significantly lower than demineralized groups ($p<0.05$); but higher than sound ($p<0.0001$). Within the remineralization groups, ΔQ_D was higher with increased demineralization days ($p<0.0001$).

Light-induced Fluorescence Images

Table 2 shows examples of light-induced fluorescence images during dehydration at 0, 5, and 10 seconds for all groups at three stages. At the sound stage, no differences were observed during dehydration for any group. At the demineralized stage, when the surface was hydrated/wet at baseline (0 second of dehydration), demineralized enamel was not observed. After 5 and 10 seconds of dehydration, demineralized enamel was clearly observed in the center of specimens as a darker rectangular shape. As demineralization time increased, demineralized enamel got darker. At the remineralized stage, the same phenomena were observed. However, remineralized enamel showed lighter color than demineralized enamel.

Microfocus Computed Tomography

After demineralization, lesion volume (μm^3) computed using the µ-CT images was numerically increased for all groups, with the trend of lesion volume increasing as demineralization time was increased. After remineralization, the lesion volume was numerically lower than the demineralized enamel but higher than sound enamel. No statistically significant differences were found ($p=0.14$); however the sample sizes for these measurements were smaller than those for the other study outcomes. The correlations of lesion volume with reflection, roughness, and ΔQ_{10} were -0.63, 0.71, and 0.82, respectively ($p<0.0001$).

Microfocus Computed Tomography Images

Table 3 presents examples of 3D images of the µ-CT for all groups. At the demineralized stage, lesion area clearly showed as dark gray color. After remineralization, lesion area decreased, especially in the Demin 3d group. In integrated images, pink/red colors indicate lesion area at the demineralized stage and green color indicates lesion area at the remineralized stage. Figure 3 shows an example of 2D cross-section images of the µ-CT from the Demin 9d group. After remineralization, we were able to observe a distinct surface layer being formed on the specimens, which was thicker than at the demineralization stage.

Discussion

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An active lesion is a caries lesion that exhibits evidence of progression for a specific period of time. An inactive/arrested lesion is a caries lesion that exhibits no evidence of progression for a specific period of time. There are several reports on the characteristics of caries lesion activity in the literature [22 – 26]. Determination of caries lesion activity, whether a caries lesion is active or inactive/arrested, is a critical component of appropriate caries diagnosis, because incorrect diagnosis may easily result in incorrect treatment decision. Active caries lesions will require either surgical or nonsurgical treatment. On the other hand, inactive/arrested lesions may not require immediate or any treatment. Incorrect diagnosis as an active caries lesion, which the lesion should have been classified as inactive, may result in unnecessary surgical treatment. Although a high reliability for assessment of caries activity was shown with criteria based on observations of specular reflection by visual assessment and texture by tactile sensation [25 – 27], previous studies also highlight the difficulty in using subjective assessments of caries lesion activity [28, 29]. Therefore, there is a need to establish a relationship of the dynamic caries process (demineralization and remineralization) with objective and quantitative measurements, such as reflectometry for visual assessment and profilometry for tactile sensation.

Reflectometry

We evaluated the relationship between specular reflection and caries lesion activity using optical reflectometry. It was expected that the demineralized enamel surface would present less reflection than sound enamel as the surface was demineralized. It was also expected that the remineralized enamel surface would present higher reflection than demineralized enamel as the surface was remineralized. The results of current study demonstrated that after demineralization, reflectance was decreased for all groups significantly, then after remineralization, it was increased significantly for all groups compared to demineralization. Thus, our hypothesis was supported by the results. There was also a strong correlation between lesion volume and reflection; larger mineral loss was associated with less reflection. Using natural non-cavitated lesions, similar results showed that perpendicular reflection intensity of visually assessed active lesions was lower than that of visually assessed inactive lesions and control sound sites [30]. Our results indicated that after remineralization, reflection would gain, although the values of remineralized enamel were similar to these of sound enamel. These results confirm that active caries lesions appear dull and inactive lesions appear to be shinier, closer in appearance to a sound surface.

Profilometry

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We evaluated the relationship between surface roughness and caries lesion activity using surface profilometry. It was expected that after demineralization, roughness would increase, then after remineralization, roughness would decrease compared to demineralized enamel. The results showed that after demineralization, roughness was significantly increased for all groups. After remineralization, roughness was slightly larger than demineralization, except Demin 6d-Remin group, which was significantly higher than demineralized group. Roughness of remineralized enamel was significantly larger than that of sound. Thus, our hypothesis for roughness was partially accepted. Additionally, among demineralized groups, roughness was smaller as demineralization time increased, although there was no significant difference. Other studies found similar results. One study indicated a large increase in surface roughness after the 30 seconds of buffered acid exposure; however, longer acid exposure did not increase surface roughness. In fact, a slight decrease in surface roughness had been observed [31]. It was suggested that this phenomenon is due to the precipitation of a new calcium phosphate phase on the surface. Similar results were found in a later study indicating that during demineralization there was a saturating exponential with increased time followed by a plateau [32]. However, in our current study, after remineralization, roughness got slightly larger; but there was no statistically significant difference. There is no clear explanation of this phenomena. This may suggest an additional precipitation on the surface created rougher surface; however, average difference was 0.31 µm and there were no significant differences. As shown in Table 3, after remineralization, lesion area/volume was decreased considerably, especially for Demin 3d group (Table 1). It is speculated that precipitation of calcium phase might be occurred after remineralization as previous studies have suggested [31, 32]. Additional support is provided in Figure 3, with the formation of a distinct surface layer after the remineralization phase (Fig. 3B), which was clearly thicker than at the demineralization phase (Fig. 3A). This phenomena appeared to be more pronounced as demineralization time was shorter. This precipitation may have closed microchannels to create a smoother surface. This may be the reason for increasing specular reflection. There was strong positive correlation between lesion volume and roughness. Another study supporting this phenomenon demonstrated that there was a correlation between the degree of the enamel surface roughness and the depth of the natural carious lesions [33]. Based on the above mentioned roughness and reflection data, our fundamental hypothesis was partially accepted.

Light-induced Fluorescence: Severity Assessment

Fluorescence intensity after 10 seconds of dehydration should represent the stable dehydrated stage [13, 15]. Therefore, ΔQ values at 10 seconds of dehydration (ΔQ_{10}) can be used to

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determine severity of caries lesions. Our results demonstrated that after demineralization, ΔQ_{10} was increased for all groups significantly. Among the demineralization groups, ΔQ_{10} increased with additional demineralization days. After remineralization, ΔQ_{10} was significantly lower than demineralized enamel, except Demin 9d (9 days demineralization). Within the remineralization groups, ΔQ_{10} was higher with increased demineralization days. In addition, there was very strong positive correlation between lesion volume and ΔQ_{10}. Several other studies have also supported the ability of this fluorescence technique to quantitatively and objectively monitor the caries process transitions from a sound surface to demineralization and then remineralization [13, 34 – 36]. The results of our current study are supported by these studies confirming that this fluorescence technique can quantify enamel de- and remineralization.

Light-induced Fluorescence: Activity Assessment

In this current study, after demineralization, change-in-ΔQ per second (ΔQ_D) was significantly increased for all groups. After remineralization, ΔQ_D was significantly lower than demineralized enamel, but higher than sound enamel. Within the remineralization groups, ΔQ_D was higher with increased demineralization days. During dehydration, the rate of vaporization of water in the lesion body should be faster for an active caries lesion than for an inactive/arrested caries lesion due to optical changes in the lesion. This change results in more pronounced light scattering in active caries lesions than in inactive/arrested caries lesions. As a result, the change-in-QLF variables per second (ΔQLF_D) for the active lesion stage presented larger values than that for remineralizing or inactive lesion stages. This current study demonstrated that the fluorescence technique when used with dehydration was able to distinguish among sound, demineralized and remineralized enamel. Thus, our hypotheses were supported by the results. van der Veen et al. [13] also demonstrated that QLF in combination with dehydration could be used to determine caries lesion activity of artificial lesions. However, that study required longitudinal fluorescence assessment in order to determine changes in lesion activity. We demonstrated in this current study that fluorescence in combination with dehydration could be used to determine caries lesion activity, using only information obtained at the time of examination. Although a threshold/cut-off of ΔQ_D to distinguish among sound, de- and remineralized enamel needs to be determined *in vivo*, ΔQ_D should be able to assess caries lesion activity objectively and quantitatively at the time of examination and not require monitoring over time. Therefore, the hypothesis of this study that the rate of vaporization of water in the lesion body during dehydration would indicate caries lesion activity was accepted.

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In summary, both reflection and roughness could distinguish between sound and demineralized enamel. Reflection was also able to distinguish between demineralized and remineralized enamel; however, it was not able to differentiate between sound and remineralized enamel. Roughness could not distinguish between demineralized and remineralized enamel, however, remineralized enamel was significantly rougher than sound enamel. Fluorescence technique (QLF) combined with dehydration was able to objectively distinguish among sound, demineralized and remineralized enamel lesions.

Conclusions

Within the scope of this study, the results indicated that there is a potential to determine caries lesion activity objectively. Specular reflection and roughness could distinguish sound and demineralized/active stages of caries lesions. Specular reflection was able to distinguish demineralized and remineralized/inactive stage of caries lesions. Furthermore, this *in vitro* study demonstrated the potential use of an objective and quantitative means for monitoring changes in caries lesion activity (demineralization and remineralization) at the time of a single examination, using real-time fluorescence imaging technology [Quantitative Light-induced Fluorescence (QLF)] in combination with dehydration.

Declaration of Interest

The authors declare that there is no conflict of interest.

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Table 3. Example 3D images of microfocus computed tomography for all groups. At demineralized stage, lesion area clearly showed as dark gray color. After remineralization, lesion area decreased, especially for Demin 3d group. In integrated images, pink/red colors indicate lesion area at demineralized stage and green color indicates lesion area at remineralized stage.

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Figure captions

Figure 1. Schematic diagram of surface dehydration hypothesis of this study at the lesion body level.

Figure 2. Average and standard error of ΔQ_D (%×mm^2/sec) at 5 and 10 seconds of dehydration. Significant differences among the groups and treatment periods ($p<0.05$) are represented by

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different superscript letters for 5 seconds of dehydration and different superscript numbers for 10 seconds of dehydration.

Figure 3. 2D Cross section of µ-CT image from Demin 9d group. (A) Demineralized surface layer. (B) Remineralized surface layer.

Table 1. Average and standard error of measurements obtained for each method. Different superscript letters represent significant differences in each method among the groups and treatment periods ($p<0.05$).

*No statistically significant differences were found for lesion volume ($p=0.14$), however the sample sizes for these measurements were smaller than those for the other study outcomes

Figr-1

Figure 1. Schematic diagram of surface dehydration hypothesis of this study at the lesion body level.

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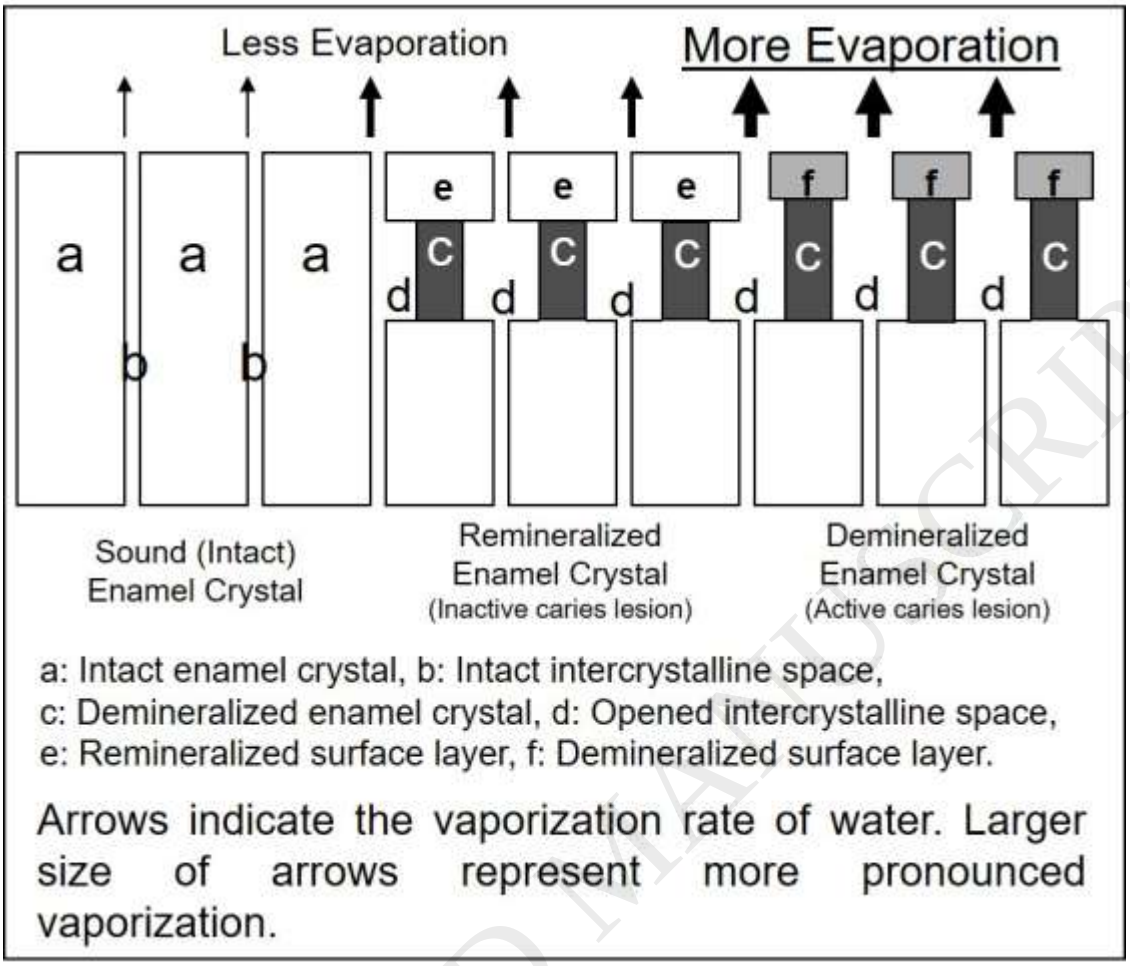

Figure 2. Average and standard error of ΔQ_D (%×mm^2/sec) at 5 and 10 seconds of dehydration. Significant differences among the groups and treatment periods ($p<0.05$) are represented by different superscript letters for 5 seconds of dehydration and different superscript numbers for 10 seconds of dehydration.

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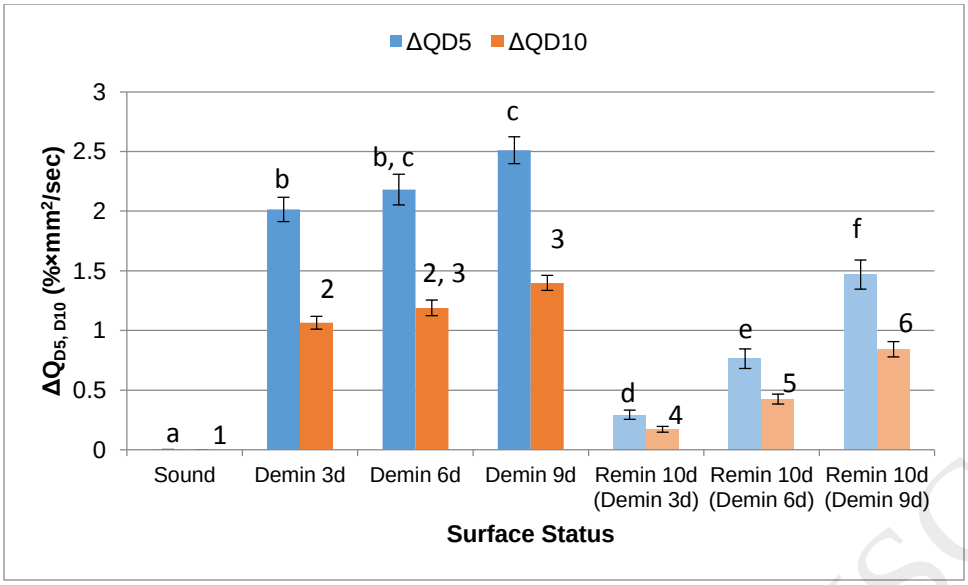


Figure 3. 2D Cross section of μ-CT image from Demin 9d group. (A) Demineralized surface layer. (B) Remineralized surface layer.

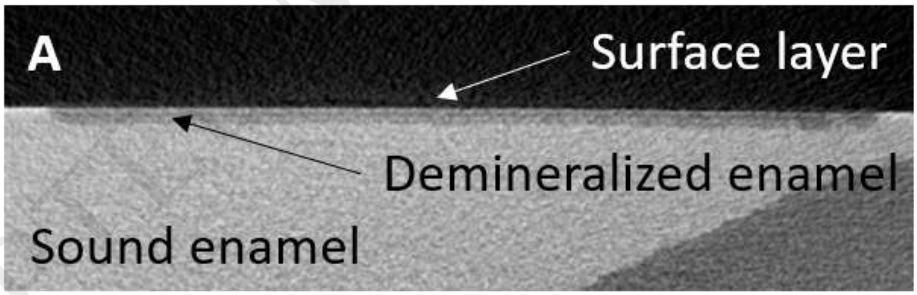
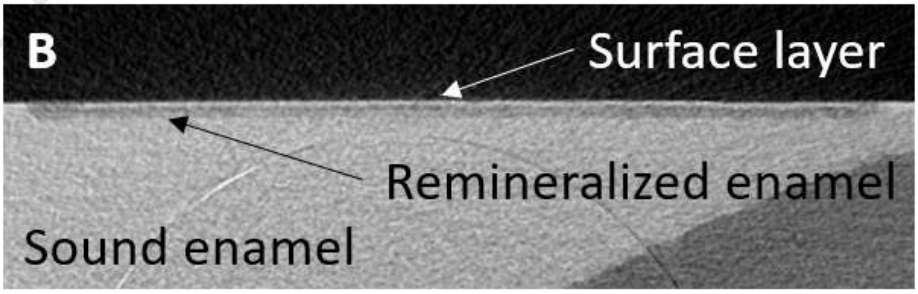
Surface Status	2D Cross section of μ-CT image
Demineralized	
Remineralized	

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Table 1. Average and standard error of measurements obtained for each method. Different superscript letters represent significant differences in each method among the groups and treatment periods ($p<0.05$).

*No statistically significant differences were found for lesion volume ($p=0.14$), however the sample sizes for these measurements were smaller than those for the other study outcomes

Method	Group	Sound	Demineralized	Remineralized
Reflection (Amplitude: %)	Demin 3d	99.7 (0.4) [a]	74.2 (1.6) [b]	100.1 (1.3) [a]
	Demin 6d		90.5 (1.4) [c]	97.4 (1.2) [a]
	Demin 9d		82.6 (1.3) [d]	98.6 (1.1) [a]
Roughness (Ra: µm)	Demin 3d	0.51 (0.08) [a]	1.82 (0.22) [b, c]	2.28 (0.29) [b, c]
	Demin 6d		1.33 (0.19) [b]	1.53 (0.21) [c]
	Demin 9d		1.28 (0.16) [b, c]	1.54 (0.19) [b, c]
Fluorescence (ΔQ_{10}: %×mm^2)	Demin 3d	0.02 (0.01) [a]	10.79 (0.53) [b, f]	1.92 (0.25) [d]
	Demin 6d		11.97 (0.67) [b, c]	4.59 (0.45) [e]
	Demin 9d		14.37 (0.66) [c]	9.08 (0.70) [f]
µ-CT * (Lesion volume: ×10^{-3} mm^3)	Demin 3d	1.83 (0.44)	15.60 (1.30)	2.87 (0.81)
	Demin 6d		22.86 (4.92)	6.67 (1.64)
	Demin 9d		25.01 (2.22)	10.28 (1.98)

Table 2. Example images of light-induced fluorescence during dehydration at 0, 5 and 10s for all groups at three stages: sound, demineralized and remineralized enamel.

Surface Status	Group	Baseline (0s dehydration)	5s dehydration	10s dehydration
Sound	Demin 3d			
	Demin 6d			
	Demin 9d			
Demineralized	Demin 3d			

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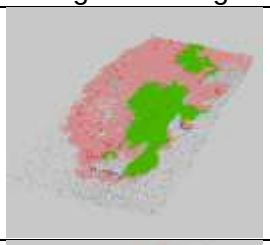
	Demin 6d			
	Demin 9d			
Remineralized	Demin 3d			
	Demin 6d			
	Demin 9d			

Table 3. Example 3D images of microfocus computed tomography for all groups. At demineralized stage, lesion area clearly showed as dark gray color. After remineralization, lesion area decreased, especially for Demin 3d group. In integrated images, pink/red colors indicate lesion area at demineralized stage and green color indicates lesion area at remineralized stage.

Group	Demineralized stage	Remineralized stage	Integrated Image
Demin 3d			
Demin 6d			

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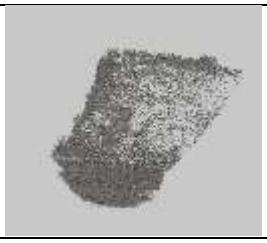
Demin 9d			

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