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Determination of Caries Lesion Activity: Reflection and Roughness for Characterization of Caries Progression

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Clinical Relevance

The results from this study indicate that reflection and roughness of a surface can be used for determination of noncavitated caries lesion progression. As caries lesions progress, surface appearance becomes duller and surface texture rougher.

SUMMARY

Caries lesion progression is difficult to determine with visual and tactile examinations. The hypothesis of this study was that reflection and roughness measurements could determine caries progression. Ground/polished sound human enamel specimens were analyzed at baseline (sound) and after two four-day demineralization periods for reflection using optical reflectometry (ORef) and for roughness using optical surface profilometry (SPro). Specimens were demineralized using a microbial–*Streptococcus mutans* caries model. Comparisons among the periods for ORef and SPro were performed using repeated measures analysis of variance. Two-sample *t*-tests were used for differences in transverse microradiography. The integrated mineral loss and depth of the four-day demineralization period were significantly smaller than those for the eight-day demineralization period ($p<0.01$). With increased demineralization time, reflection was significantly decreased and roughness was significantly increased ($p<0.01$). Correlation between ORef and SPro was moderate ($r=-0.63$). Both reflection and roughness can be characterized for nondestructive longitudinal assessment of caries lesion progression.

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INTRODUCTION

Noncavitated caries lesion activity can be defined as follows: A lesion that continues to demineralize is described as an active caries lesion, and a lesion that has stopped further demineralization is referred to as an inactive or arrested caries lesion.[1] Since caries can be arrested or reversed at their early stages, it is not sufficient to simply identify the severity of caries lesions. We know some of these lesions will progress

and therefore are in need of some form of intervention, while others may be scars of past damage (inactive/arrested) and therefore will not require any intervention. In order to select an appropriate treatment modality and aid in the clinical decision-making process, caries diagnosis and assessment of caries lesion activity are of critical importance. An incorrect diagnosis will result in incorrect treatment decisions, particularly with respect to irreversible treatments, such as dental restorations.

Visual and tactile examinations are the most common/traditional methods. Prior reports suggest that surface reflection and texture characterize carious lesion activity, with chalky and rough surfaces being active and smooth, shiny, and hard surfaces being inactive.[2-6] Based on this criterion, several studies were conducted. A study[7] showed that the intraexaminer unweighted kappa values for caries activity assessment had a wide range from 0.31 to 0.61 for ICDAS II criteria[8] plus the Lesion Activity Assessment system[5] and from 0.36 to 0.51 for Nyvad criteria.[3] ROC analysis showed that the devised classification system for determining lesion activity had acceptable accuracy (area under curve=0.84).[5] Activity kappa was in the poor to good range.[9] When visual and tactile examination was combined with the information of biofilm/plaque, the intraexaminer agreement (weighted kappa) was 0.61 and sensitivity/specificity were 0.78/0.40.[10] A study indicated that three dental examiners were unable to differentiate between the appearance (visual and tactile) of inactive lesions and active lesions.[11] Another study also showed the inability of experienced examiners to distinguish roughness by tactile examination; although examiners could repeat their own scores, they were not consistent with each other.[12] Although surface reflection and texture characterize the carious lesion activity, previous studies highlight the difficulty in using subjective assessments of caries lesion activity. There are limited reports available, especially with controlled laboratory specimens, on objective measurements of both reflection and roughness in relation to severity of demineralization (lesions). Therefore, there is a need to establish a relationship of the dynamic caries process with objective and quantitative measurements, such as reflectometry, profilometry, and histology. The hypothesis of this study was that enamel demineralization causes diffuse reflection and a rough surface. Longer demineralization times amplify these phenomena when a surface is dried. Therefore, the aim of this study was to determine whether the measurements of tooth surface reflection and roughness could distinguish severity and activity of enamel demineralization objectively.

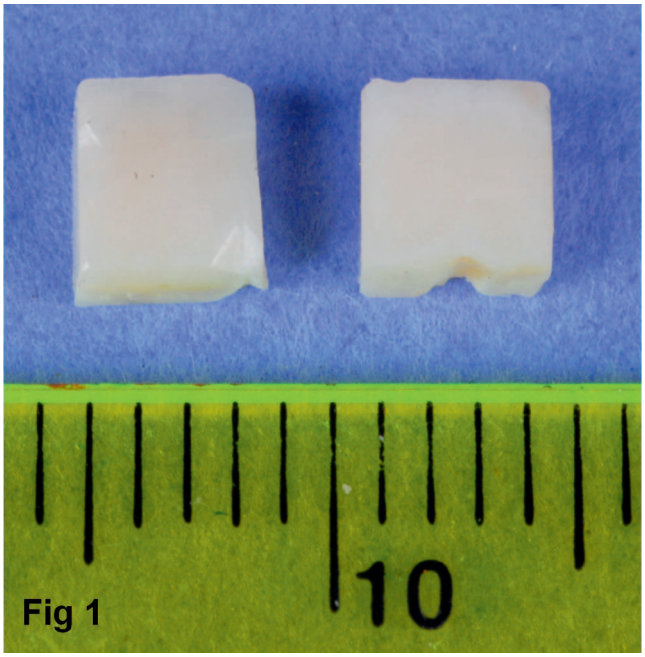


Figure 1. *Image of specimen.*

METHODS AND MATERIALS

Specimen Preparation

Extracted human teeth were collected from dental practitioners in the state of Indiana and transported in 0.1% thymol solution to the Oral Health Research Institute, Indiana University School of Dentistry. The collection of human teeth for use in dental laboratory research studies has been approved by the Indiana University Institutional Review Board. Sound permanent incisors were sterilized with ethylene oxide gas. From these teeth, 25 3 × 3 × 2-mm blocks were prepared (Figure 1). First, the enamel side was ground to establish a flat surface, and the secondary dentin side was ground by RotoForce-4 and RotoPol-31 (Struers Inc, Rødovre, Denmark). The exposed 3 × 3-mm enamel surface was polished by RotoForce-4 and RotoPol-31 until the total height was 2.0 mm (2000 μm). On the dentin side, notches were made for orientation of the specimen. While the specimens were not in use, they were kept in a humid and cold environment (4°C).

Characterization of Sound Enamel

Optical Reflectometry (ORef)—Specimens were placed inside the slot of a custom-made specimen holder. Reflectivity was measured with 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 a ground and polished human incisor tooth, with sound enamel as reference material prior to analysis. This system consists of a fiber-optic spectrometer (AvaSpec-2048, Avantes) that measures in the 200- to 1100-nm range and a 2048-pixel CCD detector. It also consists of a tungsten halogen light source (AvaLight-HAL, Avantes), which has a wavelength range of 360 to 2000 nm and optical power of 0.5 mW with 200-μm fiber. A fiber-optic reflection probe (FCR-7UV100-2-1.5×100, Avantes) was placed perpendicular to the specimen surface using a special angled fiber holder (AFH-15, Avantes). This fiber-optic reflection probe has six illuminations around one read of 200-μm fibers and a usable transmission wavelength range of 200 to 1100 nm. The reflectivity was obtained in the 380- to 780-nm range. This range was chosen based on the preliminary data that showed negligible fluctuation of sound-surface reflection. Average reflectance (%) was determined for each specimen.

Optical Surface Profilometry (SPro)—All specimens were subjected to scanning with computer-guided optical profilometry (Proscan 2000A, Scantron Industrial Products Ltd, Taunton, UK). This uses 50 W of halogen as a light source. The light is focused onto the measurement surface as a spot approximately 4 μm in diameter. The scanning frequency was 100 Hz, with a step size of 0.01 mm for the X-axis and of 0.01 mm for the Y-axis and a resolution of 0.01 μm. The center of the surface (scan area: 1.0×1.0 mm) was scanned for each specimen. The profilometric data were digitally recorded and processed by dedicated software (Proscan 2000 version 2.0.17). In this study, the parameters were limited to arithmetical mean roughness, R_a (μm), which is the most commonly used surface roughness parameter, defined as the mean deviation of the profile from the centerline, where the centerline (sometimes called mean reference line) is derived from the profile by filtering out its short-wavelength components. Ten profiles were recorded for each scan area, and mean values for each specimen were obtained by averaging them.

Demineralization

The specimens were demineralized by placing them in an *in vitro*, microbial caries model.[13] The specimens were inoculated with a mid–log phase culture of *Streptococcus mutans* A32-2 (absorbance of 0.5 at 540 nm) in trypticase soy broth without dextrose, supplemented with 5% sucrose (TSBS). After implantation, the specimens were placed in a caries-forming vessel and exposed to circulating TSBS solution for 30 minutes three times per day and to circulating mineral wash solution for 22.5 hours per day. The circulating fluids were delivered and removed from the treatment vessel by a peristaltic pump regulated by a timer. The specimens were treated for a total of eight days. The ORef and SPro data were obtained at day 4 and day 8 of bacterial demineralization, respectively. Based on a pilot study (unpublished) and previous studies,[14,15] four days and eight days of demineralization were chosen. Specimens were sterilized with ethylene oxide gas in between demineralization prior to bacterial exposure.

Characterization of Demineralized Enamel

The ORef and SPro data were obtained at four days and eight days of bacterial demineralization as described previously. At the end of each demineralization, five specimens were utilized for transverse microradiography (TMR) analysis for model validation.

Model Validation

Depth and mineral loss of specimens were determined by TMR. One section (100 μm thick) was cut from each selected specimen through the center of the specimen. All sections were mounted together with an aluminum step wedge on a glass plate (High Resolution UF Plate, Microchrome Technology, Inc, San Jose, CA, USA) and exposed to Cu(Kα) X-rays at 20 kV and 30 mA for 65 minutes. The glass plates were developed accordingly. The plates of TMR were examined with a customized computer program (Transverse Micro-radiography, Inspektor Research Systems BV, Amsterdam, Netherlands). The following data were recorded for each section: 1) depth (D [μm]) of each demineralized enamel to 83% mineral (95% of mineral in sound enamel) and 2) difference in mineral composition between remineralized and sound enamel (integrated mineral loss [IML] or ΔZ [vol%×μm]).

Data Analysis

Comparisons among the sound and demineralization times for differences in ORef and SPro were performed using repeated measures analysis of variance (ANOVA). Comparisons among the demineralization times for differences in TMR IML and lesion depth were performed using one-way ANOVA. The ranks of the measurements were used in the

Table 1: *Average and Standard Deviation of Transverse Microradiography*

Demineralization Time (Days)	Depth (μm)	Integrated Mineral Loss (vol%×μm)
4	16.1 ± 5.3	310 ± 154
8	30.7 ± 3.8	950 ± 159

analysis. Spearman (nonparametric) correlation coefficients were calculated to evaluate the associations between measurements. The correlation calculations treated each time point as independent data, ignoring the within-specimen correlations over time.

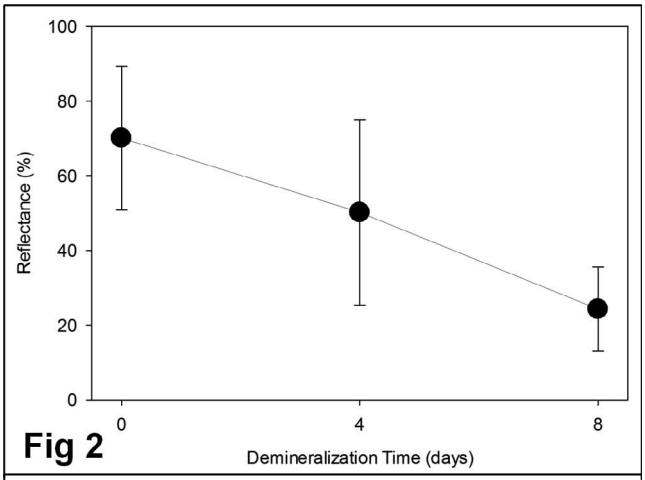
RESULTS

The results of TMR indicate that the IML and depth of four-day demineralization were significantly less than those of eight-day demineralization ($p<0.01$, Table 1). With increased demineralization time, reflection was significantly ($p<0.01$) decreased (Figure 2), and roughness was significantly increased (Figure 3). Correlation between ORef and SPro was moderate ($r=-0.63$). TMR parameters showed weak correlation with ORef ($r=-0.26$ for IML and $r=-0.13$ for depth) and moderate correlation with SPro ($r=0.67$ for IML and $r=0.55$ for depth).

DISCUSSION

Our longitudinal data confirmed that objective reflection and roughness measurements can be used for determination of caries lesion activity, especially for caries progression. With increased demineralization time, reflection was significantly decreased, and roughness was significantly increased. That means that as caries lesions progress, surfaces appear to be dull and get rough. The presence of dull and rough lesions highlights the probability of caries lesion activity when examined by clinicians.

A proof-of-concept study was conducted to demonstrate whether reflection could be used to determine caries lesion activity.[16] Reflection was measured by the vertical reflection intensity using extracted teeth that had smooth surface caries lesions. Caries activity was assessed by Nyvad criteria.[3] The results indicated low-intensity values for active lesions and higher values for inactive lesions. Active lesion surfaces presented higher roughness values than inactive lesion surfaces. Also, active lesions had larger porosity than inactive lesions. Another study was performed with natural noncavitated caries lesions. Cross-sectional data indicated that reflection may be able to be used for determination of caries



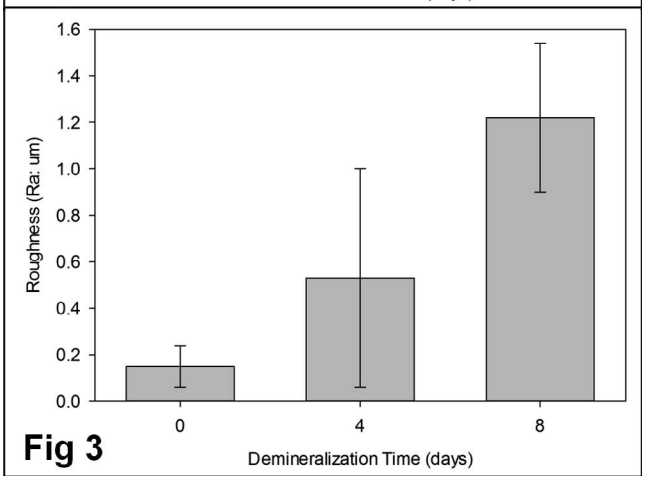


Figure 2. *Average and standard deviation of reflectance.*
Figure 3. *Average and standard deviation of roughness.*

activity.[17] Extracted teeth that had noncavitated lesions on smooth surfaces were used in this study. Caries activity was visually assessed by Nyvad criteria without probe.[3] Active lesions presented lower reflection values than inactive lesions and sound enamel surfaces. Also, active lesions presented higher roughness values than inactive and sound surfaces. A limitation of these studies was their cross-sectional design. Also, one of the studies used a total of only two specimens, one for active and one for inactive status. Advantages of our current study are utilization of a controlled microbial demineralization model and histological validation. Progression of demineralization was confirmed by histology. Despite differences between previous studies and this current study, our *in vitro* longitudinal results demonstrated the same trend to confirm these findings; as lesions progressed, reflection was decreased/lower, and roughness was increased/higher.

Sound/intact surfaces should be smoother than demineralized surfaces that have microchannels. 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.[18] This may be the reason why sound (0-hour demineralization) reflection values were higher and roughness values were smaller. Based on chemical analysis and histopathological observations, the initial stage of caries development is characterized by the opening of the intercrystalline spaces without the destruction of the surface and subsequent creation of microchannels.[19-21] Then acid penetrates from the surface into the subsurface enamel through microchannels,[19,22,23] resulting from the dissolution of the subsurface mineral. Microchannels allow the diffusion of saliva, water, and acid into the lesion body. As these microchannels enlarge, bacteria may also gain access. These microchannels are found to be about 0.5 to 1.5 μm in width in artificial lesions[19] and range from 0.2 to 1.0 μm in width in early natural enamel lesions.[24] The development of microchannels results in an irregular surface. The irregular surface reflects the light rays in various directions. This causes the surface to appear dull (nonglossy)[18] as seen in active caries lesions. Also, irregular surfaces create rough surfaces. A study using scanning electron microscopy indicated that microchannels developed at caries initiation and increased in size with continued demineralization time.[25] These may be reasons that explain that as lesions progressed, demineralized enamel showed decreased/lower reflection and increased/higher roughness compared to sound surfaces.

The results from our current study may suggest development of a handheld instrument for an objective and quantitative means to measure caries lesion activity at the time of examination (chair side). When successful, this instrument can be of great significance for clinical decision making in the management of dental caries. Particularly, both reflection and roughness measurements can be translated to clinicians with an objective and quantitative measurement for determination of caries lesion activity at the time of examination. Activity is what should drive caries interventions; therefore, objective measurement of activity will greatly facilitate clinical decision making for more effective caries management.

CONCLUSIONS

As teeth undergo continued demineralization, reflection is progressively decreased and roughness progressively increased. Both decreasing reflection and increasing roughness can be characterized for caries lesion progression. The increase in dullness and roughness of surfaces would potentially point toward active caries progression.

Regulatory Statement

This study was conducted in accordance with all the provisions, guidelines, and policies of the Indiana University Institutional Review Board. The approval code for this study is NS0911-07.

Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication. Also, there has been no financial support for this work that might have influenced its outcome.

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