Validity of a portable microhardness testing system (Cariotester) for diagnosis of progression in active caries lesions

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This study aimed to evaluate the validity of a portable microhardness testing system (Cariotester) for diagnosis of progression in active caries lesions by comparing data from this device and a laser fluorescence device (DIAGNOdent). Carious dentin in 12 specimens (stained clearly with a caries detector dye) was incrementally removed with a round bur at 150 μm depth intervals from the dentin surface in the direction of the pulp chamber. After each increment (total 138 sites), the Knoop hardness (HK) (evaluated with Cariotester) and DIAGNOdent (D) values were measured. HK values increased as D values decreased (regression formula: HK=0.419+238.342/D (p<0.001); coefficient of determination (R^2): 0.602). Although DIAGNOdent quantitatively evaluates the degree of caries progression and bacterial infection status in caries lesions, our results demonstrate the validity and convenience of alternatively using microhardness measurements during caries removal to evaluate disease progression.

Keywords: Microhardness, Laser, Fluorescence, Dentin, Caries lesion

INTRODUCTION

The concept of minimal intervention dentistry is based on evaluation and control of carious risk and caries lesion activity, and recommends observation during and after risk control, preventive and minimal restorative treatment. According to this concept, it is important to accurately and objectively diagnose the degree of caries progression in the lesion for minimal removal of dentinal tissue. However, current diagnostic methods based on visual evaluation of lesion color (with or without application of a caries detector dye) and tactile evaluation of its hardness with an excavator, are subjective. We have reported previously the design and validation of an objective evaluation method for assessing the color of carious dentin using the CIE 1976 L*a*b* color system, with which we investigated the relationship between the color of carious dentin and the presence of bacteria in the lesions in vitro.

A potential alternative diagnostic method is to measure the in vitro hardness of carious dentin, which has been attempted previously, but these studies found that the microhardness testers used these in vitro experiments were not applicable to in vivo situations. Therefore, without improved equipment, objective values of hardness in carious dentin could not be measured during removal of carious lesions in clinical situations. However, the recent development of a device for objectively evaluating the microhardness of carious dentin in clinical situations has offered new hope of pursuing this goal. This device uses a portable microhardness testing system known as the Cariotester (SUK-971, Sanei ME Corporation, Yokohama, Japan). The mechanism of this system is based on a micro-indentation hardness tester. White paint was applied to the tip of the Cariotester indenter. Under a 150 g load, this indenter was pressed into the dentin, causing paint to be lost from the portion of the indenter in contact with the dentin. The distance of paint loss ('paint-lost length') is inversely proportional to the hardness of the tissue, and these values are used by the computer to automatically calculate the Knoop hardness (HK) of the dentin. Using this system, the HK values of carious tissue can be calculated both clinically and in vitro by the same protocol. Therefore, the evaluation of microhardness may represent a novel and objective method for assessing caries lesion progression during caries removal in the clinic.

In previous studies, the in vitro measurement of microhardness in carious dentin was performed using longitudinal sections of extracted carious teeth so this technique could not be applied clinically during caries removal. Furthermore, microhardness values obtained from longitudinal sections in the laboratory are not identical to those obtained with the Cariotester from horizontal sections from dentin removal in clinical situations. This is because the differences in the surface of the carious dentin in longitudinal versus horizontal planes will affect these microhardness measurements. It is therefore necessary to investigate the relationship between dentin microhardness (assessed using the Cariotester) and the degree of caries progression in the lesion to validate the use of Cariotester for clinical estimation of microhardness.

A laser fluorescence device, marketed as DIAGNOdent (KaVo, Biberach, Germany), has been used for several years to objectively diagnose occlusal caries lesions. DIAGNOdent measures fluorescence emitted from the lesions, which a previous study has suggested originates from bacterial metabolites (e.g., protoporphyrin IX from microbes such as Prevotella intermedia and Porphyromonas gingivalis).
In addition, our previous study\textsuperscript{15,16} found that DIAGNODent evaluation in both active and arrested caries lesions was closely related to the prevalence of bacteria in those same lesions. Therefore, DIAGNOdent evaluation has potential as a tool for quantitatively evaluating the degree of caries progression in the lesion and its bacterial infection status.

In this in vitro study, we assessed the relationship between DIAGNOdent evaluation (as the standard for analyzing the degree of caries progression) and Cariotester microhardness testing in carious dentin to quantitatively evaluate the validity of using microhardness measurements to assess caries progress. The null hypothesis tested was that the use of microhardness measurements to estimate caries progress during removal of carious dentin from active lesions was not valid.

**MATERIALS AND METHODS**

**Specimens used**

Twelve specimens of coronal dentin caries (from nine extracted carious human molars) and three specimens of sound coronal dentin (three extracted non-carious human molars) were obtained from patients at the Osaka University Dental Hospital, in accordance with a protocol approved by the Ethics Committee of Osaka University Graduate School of Dentistry. These teeth were stored in physiological saline at 4°C and examined within 6 months of removal. The enamel of the carious molars was reduced with #600 grit polishing paper on a polishing machine (Ecomet III, Buehler Ltd., Lake Bluff, IL, USA) under water-cooling until the dentin caries lesion was exposed, and the color of the dentin caries lesions was evaluated. The only molars included in the experiment were those with dentin caries lesions that were pale yellow, had no natural black/brown staining or pulp exposure, and were stained clearly with the caries detector dye for 10 s, rinsed with distilled water, and air-dried with a three-way syringe\textsuperscript{7,8}. The carious dentin surface assessed with DIAGNOdent was that with the clearest dye staining. The control (sound dentin) assessed was any surface without staining. Carious and sound surfaces were then assessed 10 times with DIAGNOdent following calibration procedures. DIAGNOdent generates a laser beam through a laser diode (wavelength: 655 nm; voltage: 7.5 V; laser output: <1.0 mW), which passes through a fiber optic lead to irradiate the test dentin surface. A cone-shaped tip (diameter: 1 mm) on this fiber optic lead then measures both real-time and maximal fluorescence values (0–99, wavelength: >680 nm) emitted from these test surfaces. Calibration was performed using a ceramic standard according to the manufacturer’s instructions. The additional calibration of subtracting ‘normal’ dentin or enamel fluorescence values (obtained from sound tissue adjacent to the caries) from those of test dentinal surfaces was not carried out since the variability in the fluorescence of the sound coronal dentin in our carious samples was too high\textsuperscript{16,19}. The average of the 10 maximal values (DIAGNODent values) from each dentinal surface was calculated\textsuperscript{15,16,19}.

After DIAGNOdent assessments, the HK of the carious and sound dentin surfaces (the same surfaces as assessed with DIAGNOdent) was measured three times with a Cariotester portable microhardness testing system (Fig. 1)\textsuperscript{9}. The Cariotester consists of an indenter, a handpiece, a white paint pen and a microscope connected to a notebook computer. After application of white paint to the tip of the indenter set into the handpiece, the tip was applied to the dentin surface under a 150 g load. The paint-lost lengths on the tip of the indenter were measured using the microscope and computer (Endeavor NT2800, EPSON Direct Corporation, Matsumoto, Japan) and the HK values calculated automatically from these. Three measurements were made from each surface, with the chosen sites as central as possible in the region used for DIAGNOdent analysis, although the three indentations were necessarily separated by some distance to ensure that no measurement was influenced by a previous one made in too close proximity. Because this distribution of test sites was performed manually, it was not possible to standardize the distance between test sites. However, the distance between these indentations was determined by instead observing microscopically the paint-lost shape and degree of leaning on the tip of the indenter.
After the measurements of the paint-lost lengths. From these observations, it was estimated that the distance between indentations was generally less than half the diameter of the #5 round bur (~800 μm). The average of three HK numbers was calculated for each specimen and used for hardness evaluation.

Dentin in carious and sound specimens was removed from the test site in layers of 150 μm using a new #5 round-type steel bur (ISO configuration number: 016; diameter: 1.6 mm; around 200 rpm) under moist conditions (one drop of sterilized saline on the standard cavity preparation device). The thickness of removed dentinal tissue (150 μm) was confirmed by micrometer readings of the cavity preparation device before and after dentin removal. The application of caries detector dye to confirm clear staining of the tested dentinal sites, DIAGNOdent assessment, HK measurement and 150 μm dentin removal were repeated until 1) dentinal tissue could no longer be stained with the dye (carious specimens) or 2) until 2.0 mm of sound dentin had been removed (control specimens). In all samples, the dentin surface assessed with DIAGNOdent and Cariotester was the concave surface nearest the pulp following tissue removal with the round bur. After each dentin removal, the burs were cleaned with alcohol wipes. A new bur was used for each specimen.

**Data analysis**

For analysis of the relationship between the HK and DIAGNOdent values for all tested dentinal sites in carious specimens, we performed regression analysis with SPSS Statistics 17.0 software (SPSS Japan Inc., Tokyo, Japan). This analysis produced a regression formula, the coefficient of determination (R²) and p values for the relationship between HK and DIAGNOdent data.

For the evaluation of the accuracy of the HK and DIAGNOdent values, the interclass correlation coefficients of these values in carious and sound dentin were calculated separately using raw data (three HK numbers and 10 maximal fluorescence (DIAGNOdent) values for each tested dentin site).

**RESULTS**

From the 12 carious specimens, we obtained 138 tested dentinal sites in total for HK and fluorescence evaluations. The numbers of tested sites per specimen were distributed unevenly because each specimen had a different degree of caries progression, but these ranged from 7 to 20 sites per specimen. We also tested HK and fluorescence in a total of 37 dentinal sites in three sound dentin specimens (range: 12–13).

Figure 2 shows a representative example of the relationship between HK and DIAGNOdent fluorescence at increasing distances from the original dentin surface in a carious specimen. As this distance increased, DIAGNOdent values decreased and HK values increased. This inverse relationship was common to all tested carious specimens.

In contrast, Fig. 3 shows a representative example of a sound (control) specimen. As the distance from the original dentin surface increases, HK and DIAGNOdent fluorescence values were almost unchanged, except in the outermost layer (<0.3 mm from the dentinal surface) near the enamel-dentin junction.

Figure 4 shows the relationship between the HK and DIAGNOdent values for all tested sites of carious specimens (138 sites in total), which is shown to be an inverse relationship with DIAGNOdent values decreasing as HK increases. In caries lesions, the HK was related directly to the reciprocal of the DIAGNOdent values (D), giving a regression formula of:

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HK=0.419+238.342/D
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The coefficient of determination (R²) was 0.602, and the p value was <0.001.
DISCUSSION

In this in vitro study, we compared DIAGNOdent values with HK measurements made with the Cariotester. DIAGNOdent measures fluorescence from caries lesions that is thought to originate from bacterial metabolites, and this fluorescence closely correlates with estimates of bacterial prevalence in these lesions. DIAGNOdent is thus considered a gold standard for measuring the progression of caries. In many previous studies, bacterial culture or PCR methods have been used to evaluate bacterial infection in caries lesions. However, bacterial culture and PCR cannot be used to quantitatively analyze the amount of bacteria in dentinal tissue because the amount of dentin subjected to these methods cannot be controlled accurately. In addition, bacterial culture is extremely time-consuming. The degree of caries progression has also been evaluated by measuring dentin demineralization using contact transverse microradiography, but this is not a direct measure of bacterial infection. Because DIAGNOdent can directly detect bacterial fluorescence, it is considered a more precise method for detecting bacterial infection in caries lesions, and for objectively and quantitatively evaluating caries progress, including the status of bacterial infection. This study has defined the relationship between HK and DIAGNOdent fluorescence, and the strength of this correlation (coefficient of determination), enabling the estimation of caries progress through the use of hardness testing. This is advantageous because the DIAGNOdent system, with its cone-shaped tip measuring around 1 mm in diameter at its narrowest point, cannot accurately determine the location of the site being evaluated whereas the Cariotester, with its
sharp tip measuring around 36 µm in diameter\(^{29}\), is much more accurate. The Cariotester is therefore highly valuable for high-resolution caries diagnosis in clinical situations.

An additional advantage of using Cariotester is that the procedure is identical irrespective of whether the investigation is of an in vitro or a clinical nature\(^{30}\). Therefore, compared with previous in vitro studies using longitudinal sections of extracted teeth, in vitro data obtained using a Cariotester should more closely approximate those obtained in clinical situations\(^{31}\).

Although the tested surfaces during removal of carious or sound dentin were the macroscopically concave surfaces produced with the round bur, the small size of the tip of the indenter in relation to the diameter of the # 5 round bur means that the surface probed with the Cariotester is essentially flat. Furthermore, although the shape of the Cariotester indenter is different from that of the Knoop indenter, the relationship between the paint-lost lengths on the tip of the Cariotester indenter and the HK values was preset in the computer software accompanying the Cariotester microscope following in vitro preliminary research by the manufacturer using carious dentin from human extracted teeth. Therefore, the difference in the shapes of the Cariotester and Knoop indenters is not considered to significantly influence the measurement of HK values with the Cariotester.

The HK and DIAGNOdent values have strong interclass correlation coefficients in carious tissue, indicating high objectivity and reproducibility of these values. This validates our belief that these evaluations of microhardness and fluorescence are reliable and objective methods for assessing caries lesion progression during caries removal in clinical situations.

In this experiment, DIAGNOdent analysis was performed after application of the caries detector dye. This dye is known to influence DIAGNOdent values, with readings after dye application being larger than those before it, but this difference amounts to only around 1.08\(^{32}\). Therefore, we consider the influence of the dye on the DIAGNOdent values to be negligible in this experiment. Bacterial fluorescence and dentin structure in extracted teeth can be influenced by storage conditions and duration. The teeth used in this experiment were stored in physiological saline at 4°C and used within 6 months of extraction. Although these conditions are not excessively destructive or long, we would recommend that, in future investigations, the storage period should be minimized and the molars frozen to negate any effects of storage.

It is known that as the distance from the original dentin surface to the test site in caries lesions increases, the prevalence of bacteria in that dentin\(^{33}\) and the degree of demineralization by cariogenic bacterial metabolites decreases. Consistent with this, our data in Fig. 2 show that DIAGNOdent values (which are related to bacterial fluorescence) decrease and HK (which is related to mineralization state) increases with increasing depth from the original dentin surface. These changes appear to be mainly due to dentin caries since HK and DIAGNOdent values in sound dentin were largely unchanged except in the outermost layer, near the dentin-enamel junction (Fig. 3). This elevated hardness in the outermost layer of sound molar may be due to some residual enamel remaining after the procedures to remove it, or may represent slightly different dentin characteristics at the enamel-dentin junction. Importantly, however, in the deeper layers of dentin, HK and DIAGNOdent fluorescence are largely stable.

In active caries lesions, our data suggest a fairly strong degree of inverse correlation between the DIAGNOdent values (D) and the HK, with a coefficient of determination of 0.602 and a \(p\) value of <0.001. This suggests that, because DIAGNOdent has been shown to be related to caries progression, the HK of the dentinal tissues should also be a valid measure of caries status, including the status of bacterial infection. Therefore, we believe that taking microhardness measurements of carious dentin in active caries lesions is a valid method for evaluating caries progress.

In our previous study\(^{15}\), we found that bacteria were not detectable by PCR when DIAGNOdent gave values of <15.6 in active caries lesions. Substituting this value into the regression formula derived in this study (HK=0.419+238.342/D), this equates to a limit of bacterial detection of HK=15.7. Therefore, when HK increases above 15.7, the lesion can be predicted to be largely free of bacterial infection. Sano\(^{7}\) reported that the HK of a bacterially infected area in active caries lesions was less than 20, as determined from in vitro results of histological experiments using an optical microscope. Similarly, Torii\(^{20}\), using bacterial cultures, calculated a HK value of <17 for bacterially infected dentin. Thus, our results are in approximate agreement with previous studies.

This investigation studied only active caries lesions, which necessitates further study of arrested lesions in the future. In addition, the HK limit established (HK=15.7) for a bacteria-free lesion is likely to be rather unconservative. It is possible to tolerate a few bacteria in dentinal tissues in the long term by cavity sealing, e.g. atraumatic restorative treatment (ART)\(^{28,29}\) or sealed restoration\(^{35}\). Therefore, it will be necessary to strike a balance between complete bacterial removal and conservation of dentin tissue. This balance should take into account current and future evidence on the relationship between HK and DIAGNOdent values and the prognosis of various conservative restorative treatments.

**CONCLUSION**

We conclude that, in active caries lesions, HK is inversely correlated to DIAGNOdent fluorescence values (D). The high correlation coefficient of determination between these parameters suggests that measuring microhardness of carious dentin during removal of active caries is a valid method of
estimating caries progression. Therefore, we reject the null hypothesis that the validity of this approach was not high for evaluating caries progression, and hope to extend our research on the Cariotester and establish it as a preferred clinical diagnostic tool.

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