Relationship between Colors of Carious Dentin and Laser Fluorescence Evaluations in Caries Diagnosis

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This in vitro study investigated the relationship between assessments of dentin caries using a laser fluorescence device (DIAGNOdent) and a caries detector dye during caries removal. The dentin of eight extracted carious molars was removed at 300-μm interval points from the dentin surface toward the pulp chamber. Before and after each removal, images of the carious surfaces were taken in association with color-matching stickers (for color correction) and the surfaces were evaluated by DIAGNOdent based on fluorescence emission from the tooth surface. For the L* values (CIE 1976 L*a*b* color system), there was a strong negative correlation between DIAGNOdent results and the corrected L* values of the carious surfaces (Pearson’s correlation coefficient: r = -0.853); additionally, there was a significant correlation between them (p < 0.05). However, there were no significant correlations between the DIAGNOdent results and the corrected a* and b* values of the carious surfaces (Pearson’s correlation coefficients: 0.108 and 0.018 respectively). In conclusion, DIAGNOdent was shown to be applicable for caries diagnosis during caries removal.

Key words: Color, Laser fluorescence device, Caries diagnosis

INTRODUCTION

In clinical practice, degree of hardness and color of caries lesions are typical clinical criteria employed for caries removal[10]. However, these criteria are quite subjective and evaluations by various operators are sometimes different. Therefore, a caries detector dye has been developed for objective evaluation in clinical situations[2-5]. Generally, during removal of caries using a caries detector dye (1% acid red in propylene glycol), the dye is applied to the carious surface for 10 seconds, and stained enamel or dentin is then removed[6-7]. To avoid excessive dentin removal, dentin that is stained light pink should not be removed[5-7]. However, since the degree of light pink staining of dentin in deep carious lesions is difficult to be evaluated objectively by the naked eye, objective caries removal using a caries detector dye is not always accurate in a clinical environment[8,9].

Recently, based on diagnosis and control of carious risk in the mouth, initial caries will be further observed while progressive caries is removed minimally. This approach is based on the concept of “minimal intervention dentistry”[10,11]. Since caries removal – based on the concept of minimal intervention dentistry – is kept to a minimum, there is also a parallel need to establish better and more objective caries diagnostic methods for caries removal. As a result, objective surface analysis of caries activity or the condition of caries lesions using a pH-imaging microscope has recently been reported[12]. However, this method still requires several improvements in order to be clinically applicable.

Several years ago, a laser fluorescence device, DIAGNOdent (KaVo, Biberach, Germany), was developed for better and more objective caries diagnosis before caries removal. It has since been used in clinical situations, especially for occlusal caries diagnosis[13,14] and smooth-surface caries diagnosis[15-17] before caries removal. This device interprets the condition of the carious surface using a numerical value corresponding to the amount of fluorescence emitted by the carious surface. However, DIAGNOdent can only accurately evaluate carious dentin at the surface, but not for deep dentinal caries through dentinal tissue of carious lesions[18]. At the same time, DIAGNOdent assessment results were found to closely correlate with rates of bacterial detection in caries lesions[19]. Therefore, DIAGNOdent has potential application in the diagnosis of carious dentin during caries removal, similarly to a caries detector dye[8,20-22].

However, the relationship between the diagnostic results of the degree of caries yielded by DIAGNOdent versus those yielded by conventional diagnostic methods (such as visual inspection using a caries detector dye) has not been evaluated directly and objectively. This is because the degree of dye-stained dentin could not be clearly evaluated. To develop a more objective diagnostic method during caries removal that uses this laser device, it is necessary to investigate this relationship. In light of this necessity, we recently reported on a method that allowed objective color evaluation of carious dentin.
using numerical values\textsuperscript{33}.

Against this background of recent developments in caries diagnostic tools, this \textit{in vitro} study aimed to investigate the relationship between the results obtained from DIAGNOdent and the colors of carious dentin obtained with a caries detector dye during caries removal, using a color evaluation method of carious dentin proposed in our earlier study\textsuperscript{35}.

**MATERIALS AND METHODS**

\textbf{Measurement of color and fluorescence}

This experiment used eight extracted human molars with occlusal dentin caries. They were obtained from the Osaka University Dental Hospital in accordance with the protocol approved by the Ethics Committee of Osaka University Graduate School of Dentistry. Carious molars that were clearly stained with a caries detector dye (1% acid red in propylene glycol; Caries Detector, Kuraray, Osaka, Japan) and which did not have pulp exposure or hypoplasia were selected. Moreover, the carious molars were extracted within two days of experimental use and stored in physiological saline at 4°C. Therefore, only typical active caries were present in this experiment. This is because typical active (or acute) caries are clearly stained with the dye, while typical arrested (or chronic) caries are very weakly stained\textsuperscript{39}.

The enamel of all molars was reduced from the occlusal surface with a polishing machine (Ecomet III\textsuperscript{®}, Buehler Ltd., Lake Bluff, IL, USA) until dentin was exposed. We have previously described the method for capturing reduced dentin images\textsuperscript{34} as well as that for assessment using the laser fluorescence device, DIAGNOdent\textsuperscript{15}. Each carious molar was placed on a standardized cavity preparation device (Itoh Engineering Co., Kyoto, Japan)\textsuperscript{25}, the reduced carious dentin surface was stained with the caries detector dye for 10 seconds, rinsed with water, and then air-dried\textsuperscript{47}.

Images of the dentin surface including the color-matching stickers (Casmatch, Dai Nippon Printing Co. Ltd., Tokyo, Japan) were taken with a CCD camera (¥15; Hitachi, Tokyo, Japan). The color-matching stickers included nine colors: red, green, blue, yellow, magenta, cyan, black, grey, and white. The real \(L^*, a^*, b^*\) values (CIE 1976 L\(^*\)a\(^*\)b\(^*\) color system)\textsuperscript{26} of the nine colors were measured six times by a colorimeter (CR-100, Minolta, Osaka, Japan), and their mean values (L, a, b) were used for the next step (Table 1). Distance between the two standard light sources (Optical fiber light source PSM-11520, Nikon Corp., Tokyo; Japan; color temperature: 3100 K) and the molar test site was 10 cm, and the angle between the direction of the light sources and the surface of the test site was 45°\textsuperscript{36}. Illumination of the test site was approximately 100,000 lx as measured using an illuminometer (T-10M, Minolta).

After caries detector dye application and image capture, the reduced dentin surface of molar was measured 10 times by DIAGNOdent (this dentin test area was air-dried). This device generates a laser beam with a wavelength of 655 nm. After the laser beam is induced through the DIAGNOdent’s fiber optic lead and is irradiated the tooth substance (enamel, dentin, etc.), DIAGNOdent outputs the level of fluorescence emitted from the tooth substance. DIAGNOdent diagnoses caries on the basis that fluorescence emitted from carious surfaces is greater than that emitted from sound ones. A cone-shaped tip (diameter: 1.0 mm) attached to the DIAGNOdent was used for measurements in this experiment. DIAGNOdent displayed both the real-time and maximum values of fluorescence at the test site. Maximum values of the test sites (DIAGNOdent values) were used and evaluated in this experiment.

After image capture and measurement by DIAGNOdent, dentin in the test area was reduced in thickness by 300 \(\mu\)m from the surface under water cooling with a #5 round bur placed on a standardized cavity preparation device (around 2000 rpm), and readings in micrometers of the cavity preparation device were then recorded. From these readings before and after reduction, the relative portion of the

\begin{table}[h]
\centering
\begin{tabular}{ccc}
\hline
\textbf{Color} & \textbf{L*} & \textbf{a*} & \textbf{b*} \\
\hline
Red & 55.6±0.1 & 55.7±0.1 & 41.2±0.0 \\
Green & 65.8±0.1 & −54.1±0.1 & 29.7±0.1 \\
Blue & 39.2±0.0 & 21.4±0.1 & −43.8±0.1 \\
Black & 29.4±0.0 & −0.1±0.1 & 0.0±0.0 \\
Grey & 58.2±0.1 & −0.6±0.0 & 0.8±0.0 \\
White & 90.7±0.1 & 0.6±0.1 & −0.6±0.1 \\
Yellow & 87.5±0.1 & −14.1±0.1 & 75.9±0.1 \\
Magenta & 56.1±0.1 & 41.3±0.1 & −29.9±0.0 \\
Cyan & 63.7±0.1 & −21.2±0.1 & −30.1±0.1 \\
\hline
\end{tabular}
\caption{Mean \(L^*, a^*, b^*\) values (L, a, b) of the nine color-matching sticker colors}
\end{table}
test site was determined in the molar. Similarly, after every 300 μm reduction (from the dentin surface toward the pulp chamber of the molar) and application of the caries detector dye, images were taken and the molar’s tested dentin surface again assessed by DIAGNodent. These repetitious steps were halted when unstable sound dentin was encountered.

Dentin color correction
Methods for color correction have been described previously. Images of the test sites (i.e., reduced dentin surfaces in each molar) were transferred to a computer (iBook Special Edition, Apple Computer Inc., Cupertino, California, 95014, USA). The L*, a*, and b* values of the test sites and color-matching stickers were measured six times using Adobe Photoshop ver. 5.0 (Adobe Systems, San Jose, CA, USA). Next, mean values of the test sites (L1, a1, b1) and color-matching stickers (L2, a1, b1) were calculated. The relationship between real L*, a*, b* (L, a, b; measured with a colorimeter) and L1, a1, b1 using an approximate polynomial formula with a 3 × 3 matrix was defined according to Formula (1) as follows:

\[
\begin{pmatrix}
  L \\
  a \\
  b 
\end{pmatrix} =
\begin{pmatrix}
  g_{11} & g_{1a} & g_{1b} \\
  g_{2a} & g_{2a} & g_{2b} \\
  g_{3a} & g_{3a} & g_{3b} 
\end{pmatrix}
\begin{pmatrix}
  L_1 \\
  a_1 \\
  b_1 
\end{pmatrix}
\]

(1)

Elements of this matrix \((g_{ij}, ... g_{ib})\) were calculated by a least squares method using L, a, b and L1, a1, b1 of the nine colors of the color-matching sticker.

The relationship between L*, a*, and b* values of the test sites before color correction (Ln1, an1, bn1) and the corrected L*, a*, and b* values of the test sites (Ln2, an2, bn2) was defined according to Formula (2) as follows:

\[
\begin{pmatrix}
  L_{n2} \\
  a_{n2} \\
  b_{n2} 
\end{pmatrix} =
\begin{pmatrix}
  g_{11} & g_{1a} & g_{1b} \\
  g_{2a} & g_{2a} & g_{2b} \\
  g_{3a} & g_{3a} & g_{3b} 
\end{pmatrix}
\begin{pmatrix}
  L_{n1} \\
  a_{n1} \\
  b_{n1} 
\end{pmatrix}
\]

(2)

Therefore, the corrected L*, a*, and b* values of the test sites (Ln2, an2, bn2) were calculated using Formula (2) based on elements of this matrix \((g_{11}, ... g_{ib})\) and Ln1, an1, bn1. In addition, the corrected color values of color-matching stickers (Ln2, an2, bn2) were similarly calculated using the elements of this matrix \((g_{11}, ... g_{ib})\) and Ln1, an1, bn1.

Color differences \((\Delta E)\), L* differences \((\Delta L^*)\), a* differences \((\Delta a^*)\), and b* differences \((\Delta b^*)\) of color-matching stickers were calculated using Formula (3) as follows:

\[
\begin{align*}
\Delta L^* &= |L_n - L_c|, \quad \Delta a^* = |a_n - a_c|, \quad \Delta b^* = |b_n - b_c|,
\end{align*}
\]

\[
\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}
\]

(3)

Mean color differences \((\Delta E_{\text{ave}})\), mean L* differences \((\Delta L^*_{\text{ave}})\), mean a* differences \((\Delta a^*_{\text{ave}})\), and mean b* differences \((\Delta b^*_{\text{ave}})\) of the nine colors were calculated for all teeth.

Statistical analysis
Corrected color values of the test sites (Ln2, an2, bn2) and DIAGNodent values were analyzed by Pearson’s correlation coefficient and Fisher’s Z transformation. Significance level was set at 5%.

RESULTS
Table 1 shows the mean L*, a*, and b* values of the nine colors (L, a, b) of the color-matching stickers (these values were measured by a colorimeter). Table 2 indicates the mean color differences \((\Delta E_{\text{ave}})\), mean L* differences \((\Delta L^*_{\text{ave}})\), mean a* differences \((\Delta a^*_{\text{ave}})\), and mean b* differences \((\Delta b^*_{\text{ave}})\) between the colorimetric results of color-matching stickers of all teeth (Table 1) and their corrected color values.

Table 2 shows that the range of \(\Delta E_{\text{ave}}\) for different colors was 2.5±1.6 to 11.0±2.0 (mean±S.D.), and the mean was 7.1±3.6. Range of \(\Delta L^*_{\text{ave}}\) was 1.5±1.5 to 7.4±2.6, and the mean was 3.0±2.8.

For the carious molars used in this study, the corrected L* values \((L_{\text{cor}})\) increased as reduced dentin thickness (distance between the carious surface be-

Table 2  Mean color differences between real and corrected color values of the nine color-matching stickers

<table>
<thead>
<tr>
<th>Color</th>
<th>(\Delta E_{\text{ave}})</th>
<th>(\Delta L^*_{\text{ave}})</th>
<th>(\Delta a^*_{\text{ave}})</th>
<th>(\Delta b^*_{\text{ave}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>7.9±1.9</td>
<td>2.6±2.2</td>
<td>2.0±1.4</td>
<td>6.8±1.4</td>
</tr>
<tr>
<td>Green</td>
<td>4.8±2.2</td>
<td>2.4±1.9</td>
<td>1.9±1.5</td>
<td>3.1±1.7</td>
</tr>
<tr>
<td>Blue</td>
<td>6.2±1.1</td>
<td>1.9±1.5</td>
<td>4.3±1.5</td>
<td>3.3±1.6</td>
</tr>
<tr>
<td>Black</td>
<td>11.0±2.0</td>
<td>7.4±2.6</td>
<td>5.1±1.0</td>
<td>5.8±1.8</td>
</tr>
<tr>
<td>Grey</td>
<td>2.5±1.6</td>
<td>1.5±1.5</td>
<td>1.2±0.8</td>
<td>1.1±1.0</td>
</tr>
<tr>
<td>White</td>
<td>6.8±3.5</td>
<td>4.0±3.2</td>
<td>4.0±3.0</td>
<td>2.1±1.4</td>
</tr>
<tr>
<td>Yellow</td>
<td>10.9±4.0</td>
<td>2.8±2.7</td>
<td>4.0±1.4</td>
<td>9.2±3.9</td>
</tr>
<tr>
<td>Magenta</td>
<td>5.5±2.4</td>
<td>2.6±2.4</td>
<td>1.6±1.2</td>
<td>4.1±1.8</td>
</tr>
<tr>
<td>Cyan</td>
<td>8.7±2.0</td>
<td>1.6±1.2</td>
<td>3.8±1.8</td>
<td>7.3±2.0</td>
</tr>
<tr>
<td>Mean</td>
<td>7.1±3.6</td>
<td>3.0±2.8</td>
<td>3.1±2.1</td>
<td>4.8±3.2</td>
</tr>
</tbody>
</table>

(mean ± S.D.)
fore reduction and the test site) increased, but the corrected $a^*$ and $b^*$ values ($a_{t2}$, $b_{t2}$) showed no correlation with increase in distance. DIAGNOdent values decreased as reduced dentin thickness increased. The ranges of corrected $L^*$, $a^*$, and $b^*$ values in the eight carious molars (66 images) were respectively 19.6-71.8, 1.3-55.2, and $-10.4$-30.4 (minimum value-maximum value). The range of DIAGNOdent values was 4.9-99.0. On the other hand, from the results of our previous studies$^{19,23}$, $L^*$, $a^*$, $b^*$ and DIAGNOdent values of sound molars were more consistent than those of carious molars. These values did not always correlate with the reduced depth of dentinal tissue, but depended on the degree of caries.

Fig.1-3 show the relationships between DIAGNOdent values of carious molars and the corrected $L^*$, $a^*$, and $b^*$ values. The figure plots included the mean color values for each reduced image and the DIAGNOdent values for the reduced dentin surface in each carious molar. Pearson’s correlation coefficient between DIAGNOdent values and $L^*$ values was $-0.853$. The coefficients between DIAGNOdent values and $a^*$ values, and between DIAGNOdent values and $b^*$ values, were 0.108 and 0.018 respectively. Therefore, there was a strong negative correlation between DIAGNOdent values and $L^*$ values, but there was little correlation between DIAGNOdent values and $a^*$ or $b^*$ values. Further, from the results of Fisher’s Z transformation, there were significant differences between the DIAGNOdent values and $L^*$ values ($p<0.05$), but not between DIAGNOdent values and $a^*$ or $b^*$ values.

![Fig. 1](image1.png)

**Fig. 1** Relationship between DIAGNOdent and corrected $L^*$ ($L_{t2}$) values. Pearson’s correlation coefficient between the values is $-0.853$.

![Fig. 2](image2.png)

**Fig. 2** Relationship between DIAGNOdent and corrected $a^*$ ($a_{t2}$) values. Pearson’s correlation coefficient between the values is 0.108.

![Fig. 3](image3.png)

**Fig. 3** Relationship between DIAGNOdent and corrected $b^*$ ($b_{t2}$) values. Pearson’s correlation coefficient between the values is 0.018.
DISCUSSION

In this study, a caries detector dye (1% acid red in propylene glycol) was used for discrimination of caries during caries removal. The caries detector dye advocated by Fusayama easily discriminates caries compared to visual inspection without the dye in clinical situations. Therefore, caries detector dyes have since been used for diagnosis of removed caries in several studies. However, the relationship between staining with the dye and the extent of bacterial infection is uncertain. This is because the caries detector dye might not directly stain some cariogenic bacteria, but just stain dentinal structures destroyed and demineralized by bacterial metabolites. Additionally, discrimination of caries using a dye in clinical situations is sometimes subjective. To circumvent this problem of subjective evaluation, we designed a method aimed at evaluating carious dentin objectively using numerical values. With this objective method, we reported on how the color of carious dentin stained with a caries detector dye was related with the rate of bacterial detection in carious lesions. In the present study, we likewise adopted this objective method of using a caries detector dye. Then, we investigated the relationship between the results obtained from DIAGNOdent and the colors of caries obtained with this objective method to ascertain the potential of DIAGNOdent for clinical use during caries removal.

From Table 2, ΔE* ave was 7.1 ± 3.6, and ΔL* ave 3.0 ± 2.8. Conditions for image capture did not result in ideal Luther conditions; therefore, color correction only gave rise to approximately corrected values. However, the accuracy of color correction here was approximately the same as that in our previous study. Similarly, color differences with black and yellow were greater than those with other colors, as reported in our previous study. On this note, color selection of color-matching stickers and the formula used for color correction need to be further improved for more accurate color evaluations.

In the present study, there was a strong negative correlation between DIAGNOdent values and L* values (Fig. 1). We previously showed that L* values and DIAGNOdent values closely correlated with the rate of bacterial detection. Caries detector dye stains tooth structures destroyed by cariogenic bacterial acid or bacterial collagenase, while DIAGNOdent values are influenced by the amount of collagen in carious dentin destroyed by bacterial collagenase. Further, DIAGNOdent detects fluorescence produced by bacterial metabolites in carious dental tissues. Thus, results obtained with a caries detector dye and those obtained with DIAGNOdent are influenced by conditions of the dentinal structure destroyed by metabolic substances of cariogenic bacteria. In other words, a negative correlation between the DIAGNOdent value and L* value of carious dentin exists.

On the other hand, there was little correlation between DIAGNOdent values and a* or b* values in the present study. Similarly, in our previous study, there was little correlation between the a* and b* values of carious dentin surfaces and bacterial detection. The color of carious dentin stained by a caries detector dye is mainly of a red lineage. However, although the a* value represents the scale from red to green, the staining color of the dye represents the intensity scale of the red staining. As these scales are not the same, the a* value and the staining color had little correlation. Likewise, as the b* value represents the scale from yellow to blue, the b* value too had little correlation with the staining color. By contrast, since the L* value (lightness) is thought to relate to the intensity of color, this value was closely related to the intensity of the red staining. As a result, there was little relation between DIAGNOdent values and a* or b* values in comparison with L* values.

On the other hand, carious dentin can be distinguished between the inner and outer layers using a caries detector dye. The inner layer of carious dentin is reversibly denatured and not infected, but the outer layer is irreversibly denatured and infected. The results of this study showed that there was a strong negative correlation between DIAGNOdent and L* values of stained carious dentin, thereby indicating that the two layers of carious dentin can be distinguished by DIAGNOdent. From our previous studies, 0% bacterial detection rate was equivalent to an L* value of more than 60 or a DIAGNOdent value of less than 15.6. Thus, it is clear that DIAGNOdent can be used during removal of carious dentin in clinical situations, similar to the use of a caries detector dye.

Our experiments revealed that if DIAGNOdent is used in clinical situations during caries removal, objective numerical evaluations of carious dentin can be obtained. When the relationships between DIAGNOdent values and the extension of cariogenic bacteria, and between DIAGNOdent values and the prognosis for restoratives in clinical situations are investigated in the future, the results here should provide a better basis for minimal intervention in caries treatment. As for future clinical usage of DIAGNOdent, it is necessary to reduce the DIAGNOdent tip diameter, because its current size is too large for evaluation of carious surfaces. Additionally, the results obtained by DIAGNOdent may be influenced by nearby pulp tissues, since it was previously shown to be influenced by the internal condition of the dentin structure until it was less than 0.2-0.3 mm from the test surface. While further investigations with DIAGNOdent are necessary for its clinical applica-
tion, we believe that the present study is a step toward the future use of DIAGNOdent in clinical applications.

CONCLUSIONS

This in vitro study demonstrated the relationship between objective evaluation of dentin color following staining by a caries detector dye and the DIAGNOdent values of dentin during caries removal. The results showed that during caries removal, DIAGNOdent values of carious dentin correlated with the $L^*$ values of dentin from a caries detector dye. It could therefore be said that DIAGNOdent has potential clinical applicability in caries diagnosis during caries removal.

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