Surface Analysis of Dentinal Caries in Primary Teeth using a pH-imaging Microscope

Toru NIKAIDO1, Kayoko MORIYA1, Noriko HIRAISHI1, Masaomi IKEDA1, Yuichi KITASAKO1, Richard M. FOXTON2 and Junji TAGAMI1,3
1Cariology and Operative Dentistry, Department of Restorative Sciences, Graduate School, Tokyo Medical and Dental University, 5-45 Yushima, 1-Chome, Bunkyo-ku, Tokyo 113-8549, Japan
2Department of Conservative Dentistry, Guy’s, King’s and St. Thomas’ Dental Institute, Kings College London, London, UK
3Center of Excellence (COE) Program for Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone
Corresponding author, E-mail:nikaido.ope@tmd.ac.jp

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The aim of this in vitro study was to evaluate the pH value of intact and carious dentin in primary teeth using a pH-imaging microscope (SCHEM-100, HORIBA Ltd., Kyoto, Japan). Bucco-lingual cut sections of extracted human primary teeth, which had either mild or severe dentinal caries lesions, were placed on the pH-imaging sensor of the microscope. The pH values were compared statistically by Levene's test for equality of variances and Tukey HSD multiple comparison test (p<0.05). For both mild and severe lesions in primary dentin, the lowest pH values in the carious lesions were lower than those of intact dentin (6.6, range 6.3-6.9). There were statistical differences between the lowest pH value within the mild lesions (6.2, range 5.8-6.4) and that of the severe lesions (6.0, range 5.9-6.2) (p<0.05). It was concluded that SCHEM-100 was able to distinguish the pH-value distribution of intact and carious dentin in primary teeth.

Key words: pH-imaging microscope, Carious lesion, Primary teeth

INTRODUCTION

Caries diagnosis should be accurate and objective in order to distinguish it from intact tooth structures17. However, a significant amount of observer variation exists in conventional clinical examinations18. Many techniques in the detection and quantification of caries have been developed and investigated to examine demineralized or remineralized lesions3-9.

Recently, a pH-value analyzing technique has been introduced into dentistry which uses a pH-imaging microscope (SCHEM-100, Horiba Ltd., Kyoto, Japan)10. Preparation for the pH-imaging microscope does not necessitate samples to be destroyed by dissolution. A pH analysis can be performed by a simplified process of placing flat, solid samples on a semiconductor silicon sensor with photocurrent characteristics11.

The pH values of carious and intact dentin have been investigated visually and quantitatively. The pH distribution of carious dentin was shown to be lower than that of intact dentin using this pH-imaging microscope10. In addition, the pH value changes of active and arrested carious lesions in deep dentinal caries were evaluated with the pH-imaging microscope and then compared by observation using a caries detector solution19. Within an active lesion, there was a significant relationship between pH-imaging characterization and visual inspection using dye staining — although the arrested lesions were unstable and impermeable to dye. Hiraishi et al.13 analyzed the correlation between the pH value changes and mineral loss in active and arrested carious dentin. There was a significant correlation between a decrease in pH and mineral loss in active carious dentinal lesions.

In a series of previous studies10,12,13, human molars extracted from adult patients were used for pH-imaging characterization. However, there is little information on the pH of intact and carious dentin in primary teeth. Therefore, the purpose of this study was to analyze the surface of carious and intact dentin in human primary teeth using the pH-imaging microscope. The null hypothesis was that there was no difference in pH value between carious and intact dentin in primary teeth.

MATERIALS AND METHODS

Specimens preparation

Extracted human primary canines and molars with dentin caries lesions on the occlusal surface were used for this study. The deciduous teeth were extracted because they were about to exfoliate. Verbal agreement of their parents was obtained for their use in research and education. The teeth were stored frozen after extraction until the time for experimental procedure. The center of the carious lesion in the occlusal region was sliced vertically using a diamond saw (Leitz Instruments, Heidelberg, Germany) to obtain an approximately 1-mm thick sample.

From the sliced samples, the teeth were then
Fig. 1 Scheme of measurement procedure. (A) A tooth with a carious dentin lesion was sliced vertically to obtain an approximately 1-mm thick sample. (B) The sample was placed on the pH-imaging sensor for pH value measurement with SCHEM-100.

The pH values of mild and severe carious dentin were measured using SCHEM-100. The pH-imaging sensor was based on a Light Addressable Potentiometric Sensor (LAP) made of Si₃N₄/SiO₂ and silicon (Si)⁴⁰. For SCHEM-100 setup, a 1.5% agar solution consisting of 0.1 M potassium chloride and agar powder was used to make a 1-mm thick agar film on the semiconductor sensor (Fig. 1). When the dentin sample was placed on the agar film, H⁺ or OH⁻ ions traveled from the dentin surface into the agar film. The AC photocurrent varied due to the amount of H⁺ or OH⁻ ions passing through the agar film from the sample. The photocurrent was converted into grayscale pixels, with each pixel arranged for pH imaging with image analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). pH measurement was conducted at multiple points and the pH distribution displayed as pH images. As the grayscale of each pixel that made up the image was correlated to a pH value at each measurement, the pH value that composed the pH image was examined using EXCEL software (Microsoft Corp., Redmond, WA, USA)¹⁵. The spatial resolution and pH resolution of the sensor were 200 μm and 0.1 pH respectively.

The carious lesion or intact dentin was defined in the image analysis as follows: the lowest intensity of the grayscale area was determined to be carious lesion; the highest intensity, on the other hand, was intact dentin¹²,¹³.

**Statistical analysis**
The pH measurement results were statistically analyzed among intact dentin, mild and severe dentinal lesions using Levene’s test for equality of variances. Differences among the groups were assessed with Tukey HSD multiple comparison test. Statistical significance in all analyses was set in advance at 0.05-probability level using SPSS statistical package for Windows version 11.0 software (Chicago, IL, USA).

**RESULTS**
The pH values for each sample are listed in Table 1. The mean and range of the pH values of the intact and carious lesions in primary teeth are summarized in Table 2. Intact dentin areas in the samples showed a mean pH value of 6.6 (range 6.3-6.9). The lowest pH value of mild carious dentin was 6.2 (range 5.8-6.4), while that of severe carious dentin was 6.0 (range 5.9-6.2). The Tukey HSD multiple comparison test revealed a significant difference in pH values between intact dentin and mild and severe carious groups (p<0.05).

Fig. 2 is a photograph of a representative case with severe carious lesion (left) and its image with SCHEM-100 (right). The carious lesion with discoloration and pigmentation in the photograph corresponded to the grayscale change in the pH image.
Table 1  The pH values for each sample

<table>
<thead>
<tr>
<th>No.</th>
<th>Caries lesion</th>
<th>pH value</th>
<th>Intact dentin</th>
<th>Caries dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild</td>
<td></td>
<td>6.8</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>Mild</td>
<td></td>
<td>6.9</td>
<td>6.3</td>
</tr>
<tr>
<td>3</td>
<td>Mild</td>
<td></td>
<td>6.3</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>Mild</td>
<td></td>
<td>6.6</td>
<td>6.1</td>
</tr>
<tr>
<td>5</td>
<td>Mild</td>
<td></td>
<td>6.3</td>
<td>5.8</td>
</tr>
<tr>
<td>6</td>
<td>Mild</td>
<td></td>
<td>6.5</td>
<td>6.2</td>
</tr>
<tr>
<td>7</td>
<td>Mild</td>
<td></td>
<td>6.6</td>
<td>6.2</td>
</tr>
<tr>
<td>8</td>
<td>Mild</td>
<td></td>
<td>6.8</td>
<td>6.3</td>
</tr>
<tr>
<td>9</td>
<td>Mild</td>
<td></td>
<td>6.5</td>
<td>6.3</td>
</tr>
<tr>
<td>10</td>
<td>Mild</td>
<td></td>
<td>6.7</td>
<td>6.4</td>
</tr>
<tr>
<td>11</td>
<td>Severe</td>
<td></td>
<td>6.5</td>
<td>6.1</td>
</tr>
<tr>
<td>12</td>
<td>Severe</td>
<td></td>
<td>6.6</td>
<td>6.1</td>
</tr>
<tr>
<td>13</td>
<td>Severe</td>
<td></td>
<td>6.4</td>
<td>5.9</td>
</tr>
<tr>
<td>14</td>
<td>Severe</td>
<td></td>
<td>6.3</td>
<td>5.9</td>
</tr>
<tr>
<td>15</td>
<td>Severe</td>
<td></td>
<td>6.6</td>
<td>6.2</td>
</tr>
<tr>
<td>16</td>
<td>Severe</td>
<td></td>
<td>6.6</td>
<td>5.9</td>
</tr>
<tr>
<td>17</td>
<td>Severe</td>
<td></td>
<td>6.5</td>
<td>5.9</td>
</tr>
<tr>
<td>18</td>
<td>Severe</td>
<td></td>
<td>6.4</td>
<td>5.9</td>
</tr>
<tr>
<td>19</td>
<td>Severe</td>
<td></td>
<td>6.6</td>
<td>6.2</td>
</tr>
<tr>
<td>20</td>
<td>Severe</td>
<td></td>
<td>6.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 2  The pH values of intact and carious lesion in the primary teeth

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact dentin</td>
<td>6.6±0.2</td>
<td>6.3-6.9</td>
</tr>
<tr>
<td>(n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild carious dentin</td>
<td>6.2±0.2</td>
<td>5.8-6.4</td>
</tr>
<tr>
<td>(n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe carious dentin</td>
<td>6.0±0.1</td>
<td>5.9-6.2</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Superscript letters indicate significant difference (p<0.05)

Fig. 2  A photograph of a representative case with severe carious lesion (left) and its image with SCHEM-100 (right). The carious lesion with discoloration and pigmentation in the photograph corresponded to the grayscale change in the pH image.

DISCUSSION

Conventionally, the pH value of carious dentin has been examined using a glass electrode showing that it was lower than sound dentin, especially in active lesions. When a glass electrode was used, samples of carious dentin were collected and suspended, and the pH value of the suspension measured. It had been difficult to evaluate the distribution of pH values on carious lesions until SCHEM-100 was developed. Specimen preparation for SCHEM-100 is simple and does not require specimen destruction and dissolution. The pH distribution of a flat dentin surface was measured by a quantitative monitoring technique when the sample was placed on an agar film. TIFF images were easy to manipulate in order to observe the pH value distribution over an area of interest on the dentin surface.

In a previous study using SCHEM-100 for active and arrested carious lesions in permanent teeth, a comparison was made between pH value distribution and stainable lesion by a caries detector solution. While the caries detector solution failed to penetrate into arrested carious lesions, SCHEM-100 was able to distinguish active and arrested caries lesions. Hiraishi et al. performed another comparison with a pH and mineral-loss examination on active and arrested carious lesions. Mineral content over carious lesions was quantified using an x-ray analytical microscope. This method required no films and it captured x-rays transmitted through samples by a scintillation detector so that objective point analysis could be carried out over a mapped image. Lower pH and considerable mineral loss were demonstrated especially in active carious dentin, resulting in a significant correlation between a decrease in pH and mineral loss.

The pH values of intact and carious dentin in the primary teeth were measured using SCHEM-100 in this study. Since primary teeth have thinner enamel and dentin than permanent teeth, the areas for pH-image characterization in primary teeth are smaller compared to permanent teeth. However, differences between the intact and carious dentin were clearly distinguished in the TIFF images with SCHEM-100.

Intact dentin of the primary teeth showed a mean pH value of 6.6 (range 6.3-6.9). The mean pH value of the intact dentin of permanent teeth showed a neutral value (range 6.8-7.4), while the mean pH value of intact enamel showed around 8.0 (unpublished data). These results strongly suggested that mineral content in tooth structure influenced the pH value. The pH value of intact dentin in primary teeth was relatively lower than that in permanent teeth. Histologically, there are differences between the dentin of primary teeth and the dentin of permanent teeth, including differences in mineral and water content. These differences might be the reason for lower pH value in primary teeth.

The lowest pH values of both mild and severe carious dentin in primary teeth were significantly less than that of intact dentin. In addition, there were statistical differences between the lowest pH values of intact and carious dentin in the primary teeth.
value of mild carious dentin (6.2, range 5.8-6.4) and that of severe carious dentin (6.0, range 5.9-6.2) (p<0.05).

Kitasako et al.\textsuperscript{13} reported that the lowest pH values of both active and arrested lesions in permanent teeth were less than that of intact dentin. There were statistical differences between the lowest pH values of the active lesion (range 5.3-5.8) and those of the arrested lesions (range 6.3-6.6) (p<0.05). Therefore, the lowest pH values of mild and severe carious lesions of the primary teeth were in between those of the active and arrested lesions in the permanent teeth.

According to clinical and gross histological characteristics, carious lesions can be classified into three groups: active, arrested, and mixed lesions\textsuperscript{20}. Active caries is mainly found in young children or adolescents, while arrested caries usually occurs in older age groups. An arrested dentinal lesion differs from an active lesion by its darker pigmentation, absence of viable bacteria within the tubules, impermeability to dyes and isotopes, and a higher calcium content and hardness\textsuperscript{31}. The carious dentin of the collected primary teeth was probably close to arrested lesions because of dark pigmentation and a hard or leathery surface\textsuperscript{20}. The reasons for extraction of those teeth were due to deep caries and that it was time for exfoliation. Primary teeth with active carious dentin before exfoliation are usually restored for conservation.

However, these pH differences between mild and severe lesions in primary dentition suggested that there were different levels of caries activity, which could be due to differing properties of decalcification within peritubular dentin or intertubular dentin\textsuperscript{23}, denaturation of intratubular protoplasm\textsuperscript{24}, or debris from dental plaque micro-organisms\textsuperscript{21,25,26}.

In addition, the pH distribution within a caries-affected area is an important indicator of caries activity. The lowest pH point in the carious lesion of primary teeth was always detected on the inner area of the periphery of pH image characterizing carious lesion, which was the same as in previous studies\textsuperscript{12,13}. Moreover, the pH level gradually and eventually rose from the lowest pH level up to the level of intact dentin. There was apparently a balance between the destructive process and the response of the tooth\textsuperscript{20}. The lesion may never become completely arrested or it may become reactivated.

From the present study, the null hypothesis was rejected. Since this study demonstrated a continuous reduction in pH value in and adjacent to the carious lesions, the pH examination seemed an appropriate alternative method for caries detection. The pH analyzing technique was utilized only in vitro this time because the pH-imaging sensor was not designed for intraoral usage. However, with technology developments, it would soon be possible to use it as a clinical device for detection and quantification of caries lesions in vivo, and even serve as a standard for improved clinical practice.

ACKNOWLEDGEMENTS

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