

In Vitro pH Analysis of Active and Arrested Dentinal Caries in Extracted Human Teeth Using a Micro pH Sensor

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The aim of this study was to evaluate the pH at the surface of active or arrested carious dentin using a micro pH sensor, and to compare the relationship between different pH measurement techniques. Twenty extracted carious teeth were divided into two groups, active or arrested caries, according to predefined clinical criteria before extraction. The surface pH values of carious dentin were measured using three methods: surface pH directly measured using a micro pH sensor (Direct); sectioned teeth measured using a pH-imaging microscope (Imaging) or micro pH sensor (Slice). For all techniques, statistically significant differences in pH values were observed between active and arrested dentinal caries ($p < 0.05$). In addition, positive relations between the three pH measurement methods were found. In conclusion, Direct pH measurement using micro pH sensor might assist in caries lesion assessment and clinical treatment based on the concept of Minimal Intervention.

Key words : pH, Active caries, Arrested caries

INTRODUCTION

Currently, dental caries diagnosis is based on detection of lesions by subjective visual examination and intraoral radiographs, and diagnosis results have been shown to vary greatly among practitioners. This is because based on current diagnosis techniques, examination outcomes for caries lesions are highly dependent on the knowledge and skills of the dentist^{1,2}. Bitewing radiographs are commonly used as a diagnostic aid to visual inspection; however, early detection of lesions in enamel is difficult and bitewing radiographs are not useful in detecting incipient lesions^{3,4}.

Much of the research work on detection and quantification of caries has been carried out on extracted teeth^{5,6}. Diagnostic techniques based on digital radiography, laser fluorescence, and electrical conductance are used by dentists as adjuncts to visual inspection^{6,7}. Recently, the pH values of extracted carious and intact dentin have been investigated visually and quantitatively using a pH-imaging microscope⁸. Using this technique, the pH distribution formed on a thin agar film can be measured quantitatively, and a pH-dependent electric signal at each measurement point is converted and displayed as a pH image. As a result, pH distribution of carious dentin was found to be lower than that of intact dentin. In addition, there was a significant correlation between decrease in pH and mineral loss in active dentinal caries lesions. However, in its present

form, the pH-imaging sensor is too large for direct evaluation of caries pH in the oral cavity. A smaller version would allow *in vivo* pH-imaging and characterization of caries lesions in the oral cavity.

In 1958, Kleinberg⁹ developed modified types of antimony micro-electrodes for intra-oral pH measurement as a substitute for glass electrodes which were fragile. The antimony electrode could be used in many areas of the mouth because of its small size⁹. However, utilization of this micro pH sensor was unsuccessful because of the toxicity of antimony^{10,11}. In addition, the stability of pH measurements using the antimony electrode was reported to be poor^{12,13}.

Recently, a micro pH sensor using Ion Sensitive Field Effect Transistor (ISFET) has been developed to overcome the problems of both glass and antimony electrodes. For example, the ISFET sensor which we used in this study was made of a silicon semiconductor, and the surface of this sensor was composed of a layer of tantalum oxide (Ta_2O_5). Smit *et al.*¹³ reported that ISFET electrodes could record pH even from small volumes of fluid, and the results agreed with other pH-sensitive electrodes. Furthermore, this sensor also has other advantages: easy to calibrate, remains stable after calibration, can be stored dry, is durable, and has a short response time¹³. Therefore, for the purpose of measuring dental plaque pH *in vivo*, Chida *et al.*¹⁴ and Igarashi *et al.*¹⁵ did so with telemetric monitoring by having ISFET electrodes set in either orthodontic brackets or artificial teeth.

Although there are many pH profile studies concerning dental plaque on enamel¹⁶⁾, surface pH changes in carious dentin are not well understood to date. The aim of this study, therefore, was to evaluate the pH at the surface of active or arrested carious dentin from extracted teeth using a newly developed micro pH sensor, and to compare the relationship between different pH measurement techniques.

MATERIALS AND METHODS

Samples

Twenty extracted human teeth with moderate to severe dentinal caries lesions on the occlusal surface were used. Written informed consent, based on the Code of Ethics of the World Medical Association (Declaration of Helsinki), was obtained prior to subjects' participation in the study. The teeth were immediately stored frozen without any solution to keep the micro-organisms alive until the experimental procedure. Twenty samples were classified into two groups ($n=10$), active caries group or arrested caries group, by using these predefined clinical criteria before extraction: a soft surface layer for active caries, while a hard leathery surface and dark pigmentation were associated with arrested caries^{17,18)}. After extraction, the initial occlusal surfaces were photographed using a stereomicroscope (PM-10AK, Olympus Optical Corp., Tokyo, Japan) at a magnification of $\times 15$ (Fig. 1a).

Micro pH sensor

Ion Sensitive Field Effect Transistor (ISFET) sensor

(experimental manufacture, Horiba Ltd., Kyoto, Japan) consisted of a semiconductor with a flat surface (Fig. 1b), with a sensing area 0.015 mm wide and 0.75 mm long. Reference electrode (1 mm in diameter) was in contact with a small quantity of ion-exchanged water placed on the tooth surface. The ISFET sensor was monitored and controlled by a pH meter (F-53, Horiba Ltd., Kyoto, Japan) (Fig. 1c). The sample was connected electrically to the ISFET sensor and reference electrode by an electrolyte solution. In this research, the electrolyte solution was ion-exchanged water that surrounded the tooth specimen (Fig. 2). Sensor was covered with a layer of tantalum oxide (Ta_2O_5). Before measurement, pH values of the sensor were calibrated by standard solutions of pH 4 and pH 7.

pH measurement of carious dentin

The surface pH values of carious dentin were measured using three methods: surface pH directly measured using the micro pH sensor through a sponge moistened with ion-exchanged water (Direct); surface pH of sectioned teeth measured using either a pH-imaging microscope (Imaging) or micro pH sensor (Slice) (Fig. 3). From a pilot study, it was found that different surface preparation methods (600-grit, 1,000-grit silicon carbide papers and diamond polishing paste) did not affect the subsequent pH analysis. The pH readings were replicated for reliability using all pH measurement techniques evaluated in this study.

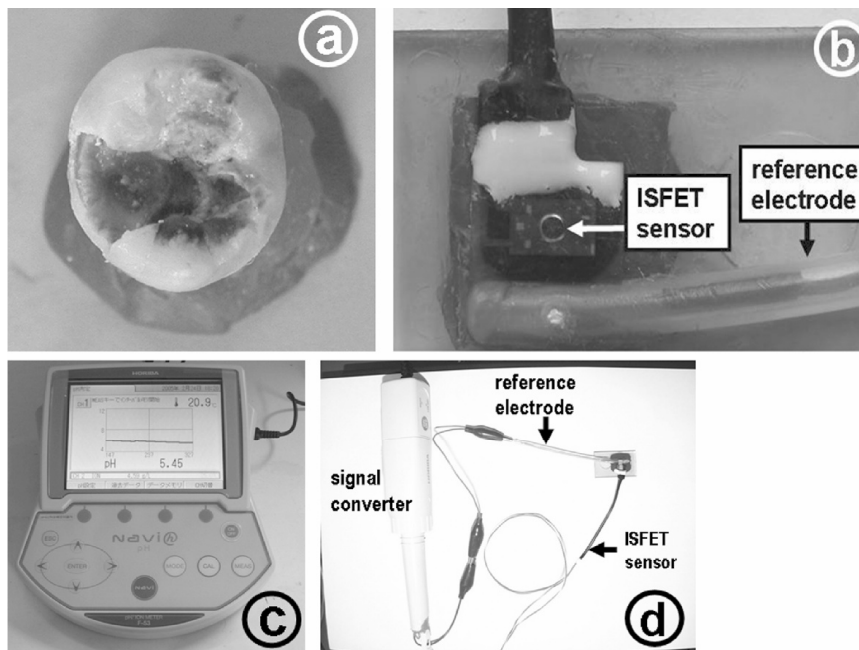


Fig. 1 Experimental setup: (a) sample; (b) ISFET sensor and reference electrode; (c) pH meter; and (d) pH measurement instrument.

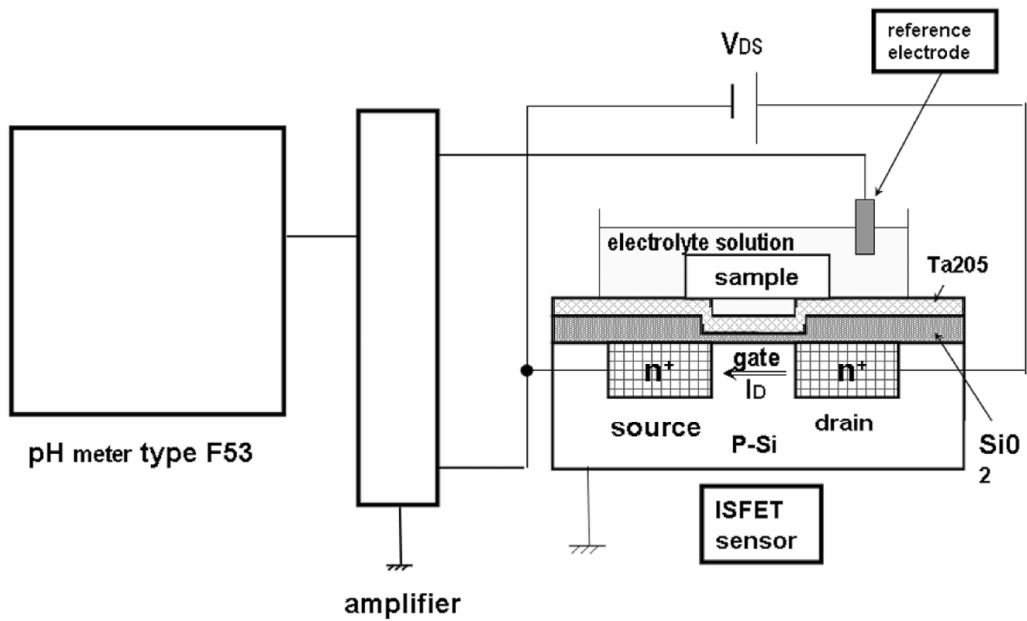


Fig. 2 Schematic diagram of micro pH measurement.

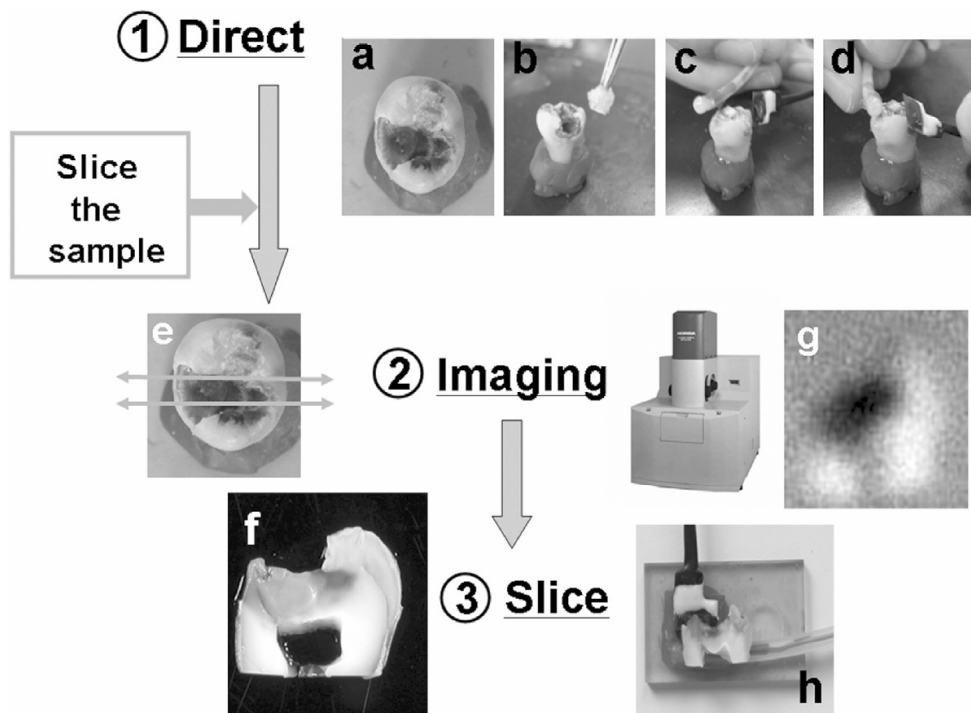


Fig. 3 Experimental procedure for the three pH measurement techniques used in this study:
 Direct method: (a) Surface pH value of extracted carious dentin was measured directly without any sample preparation; (b) Sponge was immersed in ion-exchanged water and then placed on each caries lesion; (c) and (d) ISFET sensor was placed on moist sponge, and reference electrode on a sound enamel surface via the sponge; (e) 1 mm-thick buccolingual sections were prepared; and (f) finished with wet 600-grit silicon carbide paper.
 Imaging method: (g) Surface pH of sliced sample was obtained using a pH-imaging microscope.
 Slice method: (h) ISFET sensor was placed on dental caries lesion where Imaging method showed the lowest pH value via ion-exchanged water.

Direct method

Surface pH values of extracted carious dentin were measured directly without any sample preparation (Fig. 3a). The sponge (2.8 mm × 2.8 mm × 2.8 mm; Super Bond, Sun Medical Co., Tokyo, Japan) was immersed in ion-exchanged water (pH 6.8, 0.02 g ion exchanged water per sponge), and then placed on each caries lesion for five minutes (Fig. 3b). The ISFET sensor was placed on the moist sponge, and the reference electrode on a sound enamel surface via another moist sponge to complete the electrical circuit for pH measurement (Figs. 3c and d). The pH value shown on the pH meter (F-50, Horiba Ltd., Kyoto, Japan) was then recorded to one decimal place. Before pH measurement, no effect from the sponge in terms of pH buffering was tested during pilot work.

Sample preparation for Imaging and Slice methods

Teeth were sectioned at the cemento-enamel junction with a high-speed diamond bur with water-cooling. The root was discarded and the crown portion mounted on an acrylic rod with a cyanoacrylate adhesive (Zappit, DVA Corp., Corona, CA, USA) for vertical sectioning. One millimeter-thick buccolingual sections were prepared through the central portion of the caries lesion (Fig. 3e), finished with 10 strokes of wet 600-grit silicon carbide paper, then photographed at a magnification of ×15 (Fig. 3f).

Imaging method

Surface pH of sliced sample was obtained using a pH-imaging microscope (SCHEM-100, Horiba Ltd., Kyoto, Japan) (Fig. 3g). The pH-imaging sensor was a flat semiconductor sensor with a sensing area of 2.5 cm × 2.5 cm. The sensor functioned as if it embodied an array of multiple sensing parts, as it was a single monolithic sensor with a flat sensing surface. Spatial resolution and pH resolution of the sensor were 100 μm and 0.1 pH respectively. Since this sensor could only detect protons located in the illuminated region, the single monolithic sensor functioned as if it were divided into an array of small, independent focus sensors scanning the illuminated area. The pH-dependent photocurrent at each measurement point was fed to a PC. The current was converted to a grayscale pixel, and each pixel was assigned to the pH image using an image analysis software (Image Pro Plus, Media Cybernetics, MD). In the pH image, caries lesion or intact dentin was defined as follows: grayscale area of the lowest intensity was determined to be carious lesion, while that of highest intensity was intact dentin^{8,18}.

Slice method

Before the Slice method started, sectioned surfaces were refinished with 10 strokes of 600-grit silicon carbide paper under running water. The ISFET sen-

sor was placed on the sectioned dentinal caries surface where the Imaging method showed the lowest pH value via ion-exchanged water (pH 6.8) (Fig. 3h). Reference electrode was placed on a sound enamel surface with ion-exchanged water to complete the conductive circuit. The pH value shown on the pH meter was recorded to one decimal place.

Statistical analysis

For each method, the lowest pH values of active and arrested caries lesions in dentin were compared statistically to determine significant differences by t-test ($p < 0.05$). Moreover, the correlation of pH values of both active and arrested carious lesions between Direct and Imaging, Direct and Slice, and Imaging and Slice were analyzed by Spearman Rank Correlation test. Statistical significance in all analyses was set in advance at a 0.05 probability level using SPSS statistical package for Windows version 11.0 software (Chicago, IL, USA).

RESULTS

Table 1 lists the pH values of each sample. For all techniques, there were statistically significant differences in the lowest pH values between active and arrested dentinal caries (Imaging: $p = 0.002$, 95% confidence interval for mean difference (CI for diff) = -0.469, -0.131; Direct: $p = 0.005$, 95% CI for diff = -0.481, -0.099; Slice: $p = 0.007$, 95% CI for diff = -0.523, -0.097). As for correlation of pH values between measurement methods, Spearman rank correlation analysis indicated a significant, strong positive coefficient between Imaging and Slice ($\rho = 0.890$), a significant positive coefficient between Direct and Slice ($\rho = 0.635$), and a significant moderate coefficient between Direct and Imaging ($\rho = 0.585$).

Figs. 4 and 5 are representative pH images and pH line profiles of active and arrested caries lesions, respectively. For pH-imaging analysis, the pH line profiles of both intact and carious dentin were measured. The TIFF image with pH-imaging microscope indicated a continuous pH change in and adjacent to the carious lesion.

DISCUSSION

Caries lesions may be classified according to their stage of activity. Although it is sometimes difficult to make clinical distinctions between active and arrested lesions, it is important to make this distinction as it may influence the treatment plan. The pH-imaging microscope is thus a useful tool in distinguishing between active and arrested caries lesions with respect to the lowest pH values of extracted carious dentin⁹. From the statistical analysis in this study, there was high association between the Imaging method using pH-imaging microscope and the

Table 1 The pH values for each sample

Patient age of tooth examined	Caries activity	Direct	Imaging	Slice
24	active	pH 5.3	pH 5.8	pH 5.3
28	active	pH 5.8	pH 5.9	pH 5.8
29	active	pH 5.8	pH 5.9	pH 5.7
38	active	pH 5.8	pH 5.3	pH 5.3
33	active	pH 6.0	pH 6.0	pH 6.0
52	active	pH 6.0	pH 6.0	pH 6.0
63	active	pH 6.0	pH 5.7	pH 5.9
37	active	pH 6.1	pH 6.0	pH 6.1
83	active	pH 6.1	pH 5.9	pH 6.0
40	active	pH 6.3	pH 5.8	pH 5.8
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54	arrested	pH 6.1	pH 6.2	pH 6.1
58	arrested	pH 6.1	pH 6.1	pH 6.1
64	arrested	pH 6.1	pH 6.0	pH 6.0
31	arrested	pH 6.2	pH 6.3	pH 6.1
60	arrested	pH 6.2	pH 5.9	pH 6.0
63	arrested	pH 6.2	pH 6.0	pH 6.0
67	arrested	pH 6.2	pH 6.3	pH 6.1
32	arrested	pH 6.3	pH 6.1	pH 6.1
55	arrested	pH 6.3	pH 6.1	pH 6.0
46	arrested	pH 6.4	pH 6.3	pH 6.5

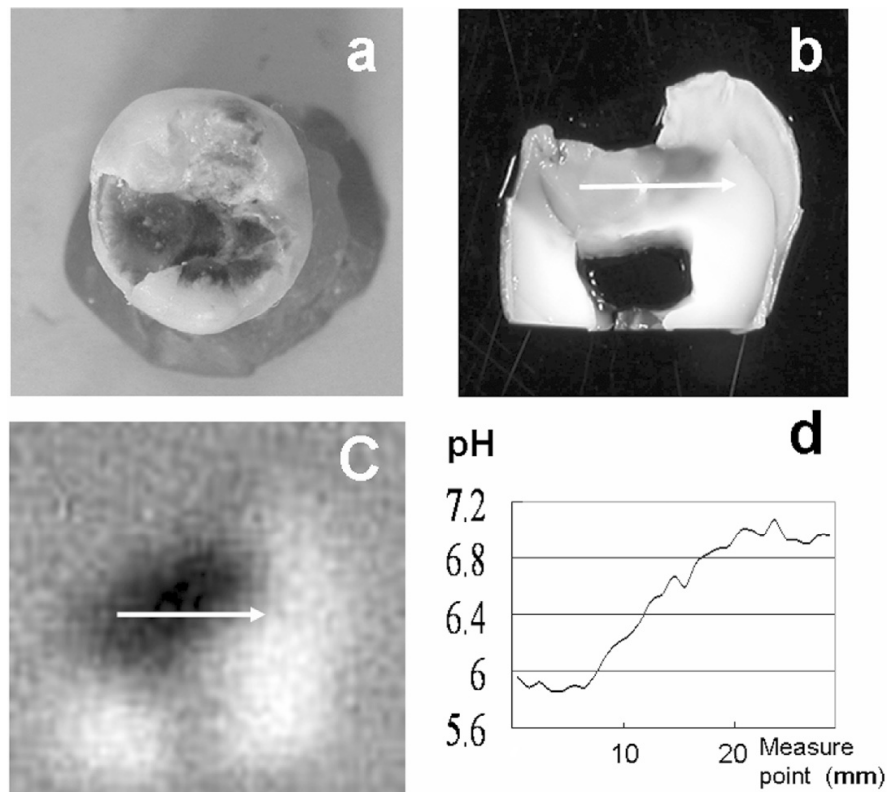


Fig. 4 Representative pH image and pH line profile of active caries (age: 28, male):
 (a) sample;
 (b) sectioned surface;
 (c) pH imaging, where grayscale area of lowest intensity was determined as the caries lesion while that of highest intensity was determined as intact tooth. pH line profile – including intact and carious dentin – was measured (arrow).
 (d) pH line profile data according to Fig. 4c (arrow).

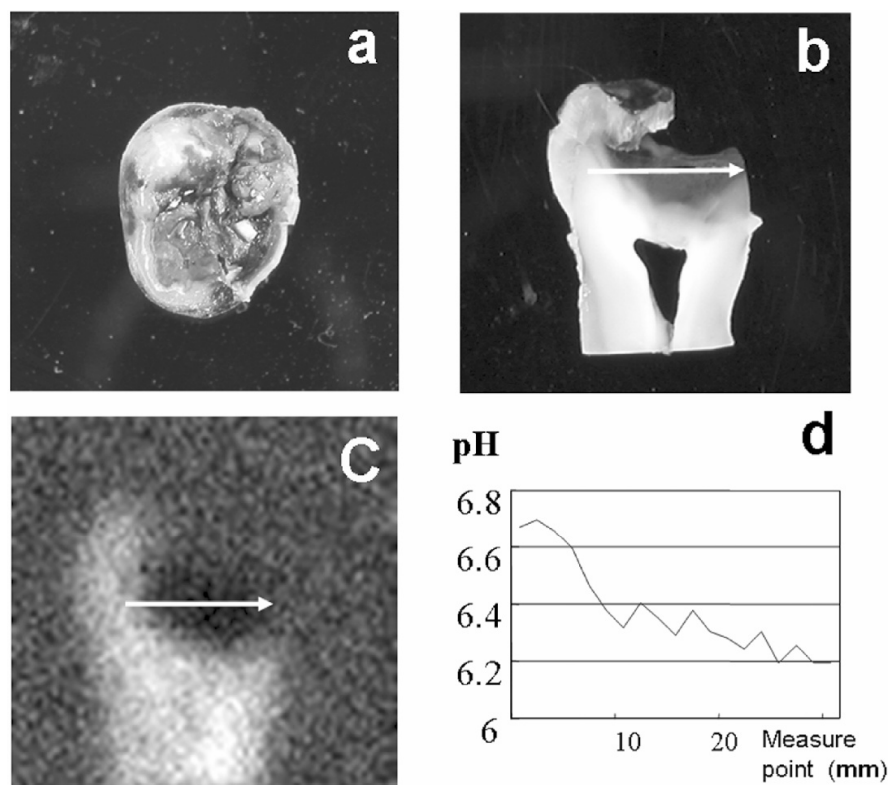


Fig. 5 Representative pH image and pH line profile of arrested caries (age: 46, male):

- (a) sample;
- (b) sectioned surface;
- (c) pH imaging, where grayscale area of lowest intensity was determined as the caries lesion while that of highest intensity was determined as intact tooth. pH line profile – including intact and carious dentin – was measured (arrow).
- (d) pH line profile data according to Fig. 5c (arrow).

Slice method using micro pH sensor ($\rho=0.890$). Therefore, it seemed the micro pH sensor could be useful too for determining active or arrested caries. However, as the sensor is flat, it can only be applied to accessible smooth surface lesions such as on incisors or root surfaces. In addition, the sensor could be used to determine the degree of acidity of plaque bacteria on individual teeth. With so many useful features, it may warrant a further development of a smaller version of this pH sensor to estimate clinical caries activity in other locations.

In this study, three different methods were used for pH measurement. Although there was a high association between the Imaging and Slice methods ($\rho=0.890$), Spearman Rank Correlation Test showed a moderate association between the Direct and Imaging methods ($\rho=0.585$). With Direct measurement, surface pH of test lesion was measured. Therefore, the pH values of the Direct method might be influenced by the morphological structure or shape of the lesion. Further, high pH values of active caries under the Direct method might be influenced by salivary

buffering. As for the Direct method setup in this study, a sponge moistened with ion-exchanged water was initially placed in each cavity for five minutes. The sponge, being soft, would vary its form according to the cavity shape. For pH analysis in the oral cavity, this technique would be very useful for assessing caries activity, particularly on individual teeth.

Based on the results obtained, there were significant differences in the lowest pH values between active and arrested caries with all the three pH measurement methods. From the Direct method results, a pH value below 5.8 could suggest an active caries lesion. However, it was difficult to distinguish between active and arrested dental caries with a pH value range of 6.0-6.3 in this study. Therefore, further studies on active and arrested caries lesions under clinical conditions are required in order to assess caries activity.

Clinical diagnoses are often based on the use of definitions and classifications of disease stages. However, this often reflects a method of convenience

rather than a variation among individuals. This absence of a clear and universally valid distinction between intact and carious tooth structure is reflected in the use of a wide variety of caries diagnostic criteria and clinical management methods¹⁹. Against this background, quantitative measurements may aid dentists to assess changes in caries activity over time. In the case of early enamel or root caries lesions, results from this pH assessment method could contribute to establishing a more individualized caries treatment plan for the patient. It would also be suitable for evaluating the remineralization effects of professional or self-care oral treatments such as fluoride mouthrinses. Further, the quantitative values may also aid in educating the patient with regard to taking control and managing his/her own state of oral health. On this note, further clinical study is needed to evaluate the surface pH of carious dentin using this micro pH sensor.

CONCLUSION

Quantitative evaluation of caries activity is believed to be an excellent means for recording an individual's caries state. Moreover, favorable outcomes from this quantitative measurement technique will contribute to providing further information for Evidence-Based Dentistry. As shown in this study, the micro pH sensor could determine the caries activity on each tooth. As such, it exhibited the potential to aid in caries lesion assessment and the subsequent clinical management plan based on the concept of Minimal Intervention (MI).

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REFERENCES

- Slack GL, Jackson D, James PMC, Lawton FE. A clinical investigation into the variability of dental caries diagnosis. *Br Dent J* 1958; 104: 399-404.
- Grondahl H. The influence of observer performance in radiographic caries diagnosis. *Swed Dent J* 1979; 3: 101-107.
- Ekstrand KR, Ricketts DN, Kidd EA. Reproducibility and accuracy of three methods for assessment of demineralization depth on the occlusal surface: An *in vitro* examination. *Caries Res* 1997; 31: 224-231.
- Ricketts DN, Kidd EA, Smith BG, Wilson RF. Clinical and radiographic diagnosis of occlusal caries: A study *in vitro*. *J Oral Rehabil* 1995; 22: 15-20.
- Brinkman J, ten Bosch JJ, Borsboom PC. Optical quantitation of natural caries in smooth surfaces of extracted teeth. *Caries Res* 1988; 22: 257-262.
- Lussi A, Imwinkelried S, Pitts NB, Longbottom C, Reich E. Performance and reproducibility of a laser fluorescence system for detection of occlusal caries *in vitro*. *Caries Res* 1999; 33: 261-266.
- Verdonschot EH, Kuijpers JM, Polder BJ, De Leng-Worm MH, Bronkhorst EM. Effects of digital grey-scale modification on the diagnosis of small approximal carious lesions. *J Dent* 1992; 20: 44-49.
- Kitasako Y, Hiraishi N, Nakajima M, Nikaido T, Tagami J, Nomura S. *In vitro* surface analysis of active and arrested dentinal caries using a pH-imaging microscope. *Oper Dent* 2002; 27: 354-359.
- Kleinberg I. The construction and evaluation of modified types of antimony micro-electrodes for intra-oral use. *Br Dent J* 1958; 104: 197-204.
- Winship KA. Toxicity of antimony and its compounds. *Adverse Drug React Acute Poisoning Rev* 1987; 6: 67-90.
- Poon R, Chu I, Lecavalier P, Valli VE, Foster W, Gupta S, Thomas B. Effects of antimony on rats following 90-day exposure via drinking water. *Food Chem Toxicol* 1998; 36: 21-35.
- Charlton G. Determination of hydrogen ion concentration in the mouth – A comparison of the glass, antimony and quinhydrone micro-electrodes. *Aust Dent J* 1956; 1: 228-232.
- Smit A, Pollard M, Cleaton-Jones P, Preston A. A comparison of three electrodes for the measurement of pH in small volumes. *Caries Res* 1997; 31: 55-59.
- Chida R, Igarashi K, Kamiyama K, Hoshino E, Esashi M. Characterization of human dental plaque formed on hydrogen-ion-sensitive field-effect transistor electrodes. *J Dent Res* 1986; 65: 448-451.
- Igarashi K, Lee IK, Schachtele CF. Comparison of *in vivo* human dental plaque pH changes within artificial fissures and at interproximal sites. *Caries Res* 1989; 23: 417-422.
- Sansone C, van Houte J, Joshipura K, Kent R, Margolis HC. The association of mutans streptococci and non-mutans streptococci capable of acidogenesis at a low pH with dental caries on enamel and root surfaces. *J Dent Res* 1993; 72: 508-516.
- Miller WA, Massler M. Permeability and staining of active and arrested lesions in dentin. *Br Dent J* 1962; 112: 187-197.
- Hiraishi N, Kitasako Y, Nikaido T, Foxton R M, Tagami J, Nomura S. Evaluation of active and arrested carious dentin using a pH-imaging microscope and X-ray analytical microscope. *Oper Dent* 2003; 28: 598-604.
- Ismail AI. Clinical diagnosis of precavitated carious lesions. *Community Dent Oral Epidemiol* 1997; 25: 13-23.