Original

Second Order Differentiation Analysis of Micro FTIR Method Revealed the Variable Erosion Characteristics of Carbonated Soft Drink for the Individual Human Teeth Enamel

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Abstract: To clarify the chemical processes of dental caries in detail, and to clarify whether or not the caries processes are common through the whole part of various teeth, the micro-FTIR spectroscopic analysis was carried out using the human teeth sections soaking into a carbonated soft drink (Sprite®) for 1 and 7 days. After the twenty human teeth examined, the teeth were grouped into two; the lightly dissolved one and extremely heavily dissolved one on the basis of the macroscopical and microscopical features after the soaking experiment. In this study, one lightly dissolved sample (A) and one heavily dissolved sample (B) were picked up and described. The micro-FTIR spectroscopy showed the drastic changes in the P-O absorption bands of the outer layer enamel of both samples, while those of the inner layer enamel remained almost unalterably. This result indicated that the erosive processes mainly attacked the phosphate ion environments in the biological apatite crystal structure of tooth enamel. The second order differential curves of the micro-FTIR patterns firstly reported here showed the small but significant P-O band peak shifts among the all analyzing points except for 7 days of Sample B, suggesting the individual physicochemical characteristic of tooth enamel apatite. This study presented only two extreme cases, but clearly showed the variety of human tooth enamel characteristics among the individuals and parts of the tooth enamel. It was suggested that generally accepted caries protection methods might not be a common standard for every people and every teeth enamels.

Key words: Human tooth enamel apatite, Carbonated beverages, Caries, micro-FTIR, Second order differential curve

Introduction

Dental caries is still the major issues to be solved in oral health care medicine^{1,2}. Along with changes in dietary life, the occurrence of dental caries due to carbonated beverages has been reported more frequently in the recent decades³. A huge number of the studies on the caries formation in vivo and in vitro had been reported, but the results of these studies are not concluded, and we have not yet revealed a unified mechanism of the caries progress^{4,6}. In spite of the principal four causes of dental caries are known such as i) tooth physico-chemical quality, ii) intake of carbohydrates, iii) activity of bacteria, and iv) passage of time, and the detection of dental teeth has made dramatic progress especially with the recent development of optical laser technology⁷, the detailed formation and progression mechanisms of dental caries have still been left to be clarified.

These conflicts may be due to the fact that the characteristics of tooth enamel are not common in macro- and micro-structural morphological ground among the individuals and the portions of enamel⁸. The biological apatites consisting the human tooth enamel showed the remarkable deviations in their crystallographic characteristics among the individuals⁹ and by the portions¹⁰. Therefore, the formation mechanism of dental caries should be studied by microscopic viewpoint, not by macroscopic viewpoint.

In this study, the variable erosive potency of carbonated soft drink for the individual human teeth enamel was studied from the chemical viewpoint using micro Fourier transform infrared (micro-FTIR) spectroscopy.

Materials and Methods

Human Tooth Samples

Twenty human third molar teeth used in this study were those extracted for dental clinical reasons and pooled in purified water. The teeth selected for this experiment were not having caries lesions but having some white and/or brownish spots. Two longitudinal sections, ca. 0.5 mm thick, were cut from the middle part of each teeth using a low-speed diamond saw (IsoMet, Buehler Co. Ltd., Lake Bluff, IL, USA).

These sections adhered onto the microscopic cover glass using a waterproofing bond (TAISUI-BOND-SOKKAN, Bijutsu Shuppan Educational Co. Ltd., Tokyo Japan). Each bonded section was put into separately a plastic mesh bag and soaked into a 1.5 l carbonated soft drink bottle, Sprite® (Coca-Cola (Japan) Co. Ltd., Tokyo Japan) for the duration periods of 1 day and 7 days. Hereafter, “1d” represented the sample soaking duration of 1 day and “7d” of 7 days. After the soaking, these samples were washed in distilled water and kept in a desiccator.

The soaked teeth were grouped into two; the lightly dissolved one and extremely heavily dissolved one on the basis of the macroscopical and microscopical features after the soaking experiment according to the preceding experiment¹¹. Among the twenty human teeth samples examined in this study, two typical samples were explained in this study.

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Figure 1. The photographs showing samples A and B: before (Left) and after (Right) the Sprite® soaking of 1 day (1d: Top) and 7 days (7d: Bottom) duration. Inset photographs are the two-times enlargements of the analyzing areas. The arrows and the numbers indicate the micro-FTIR analyzed regions. The white inset bars indicate 5 mm length. Sample A's Hunter-Schreger bands were observed remarkably, indicating the sections were subjected to the etching effect of the soaking strongly, but the outline of the tooth enamel remained as it was. Sample B's Hunter-Schreger bands were more clearly observed in the section after the 7d soaking, while not so clear in the section after 1d soaking.

Figure 2. Micro-FTIR patterns, from 1500 cm⁻¹ to 500 cm⁻¹, of the Samples A and B the soaking of 1 day (1d: Top) and 7 days (7d: Bottom) duration. The blue lines are the spectra from the outer enamel and the red lines from the inner enamel. The P-O vibration bands were observed in the Samples A 1d and B 1d. These bands were decreased or lost in the samples A 7d and the outer layer of B 7d.

Figure 3. Second order differential curves for the micro-FTIR patterns, from 1500 cm⁻¹ to 500 cm⁻¹, of the Samples A and B: the soaking of 1 day (1d: Top) and 7 days (7d: Bottom) duration. The blue arrowheads indicating the peaks from the outer enamel and red arrowheads from the inner enamel. The P-O vibration bands peaks were observed in the Samples A 1d and B 1d. These peaks were lost in the samples A 7d and the outer layer of B 7d.

Table 1. The P-O absorption band peaks (wavenumber, cm⁻¹) derived from the second order differential curves.

Sample A (lightly dissolved)				Sample B (heavily dissolved)				Hydroxyapatite		
Outer		Inner		Outer		Inner		reference	Chemical groups	Description
1d	7d	1d	7d	1d	7d	1d	7d			
<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	910	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>		
1115	1127	1120	1127	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	1020 - 1120 ⁹⁾	PO ₃ ²⁻	v3
1130	1130	1130	1130	1160	<i>n.d.</i>	1148	<i>n.d.</i>	1048, 1090 ^{**)}		

1d: 1 day duration, and 7d: 7 days duration samples.

*) see Cimdina LB and Borodajenko N¹⁴⁾

**) see Drouet C¹⁵⁾

n.d.: not detected.

The sample A was the lightly dissolved one, and the sample B was the heavily dissolved one.

This study was approved by the ethics committee of Nihon University School of Dentistry at Matsudo (approval number: 17EC-015).

Microscopic Observation

The samples before and after the soaking experiment were observed and photographed using a stereomicroscope (Leica M60[®], and Leica DFC295[®]; Wetzlar, Germany).

Micro Fourier Transform Infrared Spectroscopy (micro-FTIR)

Infrared spectroscopy is one of the oldest and well established experimental techniques for the analysis of not only organic but also inorganic substances. It is convenient, non-destructive, requires less sample preparation, and can be used under a wide variety of conditions.

The outer and inner parts of the sample A and B were analyzed by means of the micro-FTIR, especially the shift of peak position. The micro-FTIR measurement was carried out at the ST Japan Lab, and the limited number of analysis was done for the samples after the soaking experiment. A micro-FTIR instrument (Survey IR[®]; Cziitek, CT, USA equipped with a Nicolet iS5 FT-IR[®]; Thermo Scientific, Yokohama, Japan) was used in this study.

Micro-FTIR analysis was carried out under the conditions as follows: Infrared measurement: Reflection mode, Wavenumber range: 7,800 cm⁻¹ - 350 cm⁻¹, Detector: DLaTGS/KBr window, Aperture diameter: 100 μm, Resolution ability: 0.8 cm⁻¹, Gain: AUTO, Cumulated number: 64 times.

FTIR spectra analysis software was Panorama Ver4.0 Analytical Software (LabCognition Analytical Software GmbH & Co. KG.). The second differential curve was used to detect the precise peak position as a useful technique in FTIR analysis¹²⁾.

Results

Microscopic Observation

The results of the soaking in Sprite[®] experiments showed unexpectedly wide variations. Among the twenty samples used in this study, only two extreme samples were reported here, because it was sufficient to show the typical variety of the reaction observed.

Fig. 1 showed the photographs of the lightly dissolved sample A and the heavily dissolved sample B: after the Sprite[®] soaking of 1d and 7d duration, together with the micro-FTIR analyzing points. Sample A showed many white and brownish coloring spots were observed in all sections. After the soaking of 1d and 7d, Sample A's Hunter-Schreger bands were observed remarkably, indicating the sections were subjected strongly the etching effect of the soaking, but the outline of the tooth

enamel remained as it was. Sample B showed also many white and brownish coloring spots were observed in all sections. Sample B's Hunter-Schreger bands were more clearly observed in the section after the 7d soaking, while not so clear in the section after 1d soaking. The different etching aspects between the samples A and B may be due to the individual variety of the enamel properties. After the 7d soaking, the outline of the tooth enamel was greatly changed, and the portions of the central fissure and the cusps were dissolved extensively. The thickness of the sample B enamel decreases appreciably.

Comparing the samples A and B, while the erosive power was the same among the experiments, the subjected effects were greatly different, indicating the individual variety of the tooth enamel sensitivity to the acidic attack.

Micro Fourier Transform Infrared Spectroscopy (micro FTIR)

Fig. 2 showed the micro-FTIR spectra obtained from the outer and inner tooth enamel portions of the samples A and B, after the Sprite[®] soaking of 1d and 7d duration.

In Fig. 2, all the spectra of samples A 1d and B 1d, and both the outer and inner layers showed the similar pattern holistically and showed the main absorption bands at from 1200 cm⁻¹ to 1100 cm⁻¹, assigned as of the P-O vibrations in the enamel apatite crystallites.

The minor differences of these absorption band patterns at from 1200 cm⁻¹ to 1100 cm⁻¹ and from 600 cm⁻¹ to 500 cm⁻¹ (Fig.2) reflected the small environmental differences of the P-O tetrahedra and which has been extensively used to monitor the crystallinity of hydroxyapatite. The details of these configurations were not analyzed in this study, because the reflection-mode used was not sufficient to analyze these detailed structural changes. The absorption bands from 1500 cm⁻¹ to 1300 cm⁻¹ due to the B-type CO₂ in the biological apatite were observed in this study, but these bands were too weak to analyze. Therefore, the analysis for these CO₂ were not carried out in this study.

After the 7d soaking the spectra of Sample A (Fig. 2A 7d) and the spectra of Sample B outer layer (Fig. 2B 7d) showed no significant P-O absorption bands from 1200 cm⁻¹ to 1100 cm⁻¹ at all, while these P-O bands appreciably remained in the inner layers of the sample B 7d. These results indicated that the surface layers of the samples A and B tooth enamel were high sensitivity to the acid attack comparing to the inner layers.

Fig. 3 showed the second order differential curves obtained from the micro-FTIR patterns. The second order differential curves were usually calculated to detect the peak positions¹³⁾. The peak positions found in this study were listed in Table 1.

The peak positions represented in the second order differential curves of the sample A, 1115 - 1127 cm⁻¹, were almost the same within

the accuracy of the instrument. These absorption band peaks were assigned as ν_3 P-O vibrations of hydroxyapatite ($1020\text{--}1120\text{ cm}^{-1}$)¹⁴⁾, (1048 cm^{-1} , 1090 cm^{-1})¹⁵⁾. The sample A 7d did not show appreciable peaks in the second order derivative curves, and sample B 7d outer layer showed the peak shifted. The lost and the peak shift of P-O absorption band suggested the undergoing structural destruction of phosphate molecules of these enamel apatite.

Discussion

There are two major flows towards elucidating the mechanism of dental caries: i) describe and clarify its etiology and mechanism from observation of natural caries, and ii) lead a theoretical consideration by experiment with artificial caries formation. Elucidation of the formation mechanism of dental caries has been progressing slowly as compared with the recent great advancement of the method of caries detection^{7,16)}.

By a recent increase of the occurrence of dental caries due to carbonated beverages, elucidation of the mechanism of caries formation is expected more quickly and reliably according to EBM. FTIR, Fourier Transform Infrared Spectroscopy, is one of the standard quantitative and qualitative convenient, reliable method for molecule species analysis. This research is one of the few studies analyzing dental enamel by micro-FTIR⁸⁾, not by normal FTIR. In addition, the detection of peaks by second-order derivative curves¹³⁾. This is the first example of tooth enamel erosion studies. In the field of hard tissue mineralization and demineralization, a number of investigations were carried out using FTIR, and various properties of biological apatite crystals have been clarified^{17–20)}.

FTIR analysis is an extremely effective method for examining the state and arrangement of carbonate ions in biological apatite crystals. On the contrary, the study of a phosphate salt in hard tissues have been advanced greatly by FTIR; there was a long debate history about the presence and their site of the carbonate ions in the biological apatite crystal structure⁸⁾. It is now well known that the biological apatites are constituting the hard tissues, such as tooth enamel, dentin, and bone, contain the large amounts of carbonate ions at the PO₄-site, known as B-type carbonate, in the apatite crystal structure^{10, 19, 21–24)}. Moreover, it may be noteworthy that enamel carbonate levels influence caries-susceptibility^{25,26)}.

In this study, the carbonate configurations in the examined samples were not determined because of the weak absorption bands to analyze. However, instead of the Reflection Mode of FTIR used in this study, it may be able to analyze the carbonate ions by using the more precise Resolution-Enhanced FTIR which applied to bone biological apatite analysis²⁷⁾.

Micro-FTIR instrument usually has the potential to analyze a very limited region of the object as small as 10 micron-meter square. Applying this micro-analysis technique in this study, the differences of the P-O absorption bands in the FTIR patterns among the tooth enamel samples, not only between the duration periods but also between the portions of the tooth enamel, were obtained. The differences among the portions and between the samples indicated the biodiversity in tooth enamel could not be ignored. The similar variety of tooth enamel had already been reported repeatedly by many investigative approaches in the normal^{8, 10, 28–31)}, but these facts had not been described nor ignored in most oral histology textbooks.

In the concluding remarks, it may be needed for the elucidation of tooth enamel caries processes to clarify the relationship between the individual caries properties and the individual tooth enamel physicochemical diversities. This study indicated the analytical potential of reflection-mode micro-FTIR spectroscopy for these purposes.

To conduct more precise research, it will be needed to improve and develop the analytical technique and method. FTIR 2D imaging (FTIR-I) and FT-Raman with micro-X-ray diffraction techniques will be useful for the more precise study. By accumulating these fundamental research results, it can be obtained the breakthrough for dental caries prevention and promotion of remineralization that are useful for clinical practice suitable for individuals.

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Conflict of Interest

The authors have declared that no COI exists.

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