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 macroscopic 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 medicine1-2). 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 progress4-6). 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 portions8). 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 experiment10). 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 decreased or lost in the samples A 7d and the outer layer of B 7d.
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®; Czitek, 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: 4000 cm\(^{-1}\) - 700 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 analysis\(^{12}\).

**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 CO\(_2\) in the biological apatite were observed in this study, but these bands were too weak to analyze. Therefore, the analysis for these CO\(_2\) 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 positions\(^{13}\). 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 v3 P-O vibrations of hydroxyapatite (1020-1120 cm⁻¹), (1048cm⁻¹), (1090cm⁻¹) 15). The sample A 7d did not show appreciable peaks in the second order differential curves, and sample B 7d outer layer showed the peak shift. 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 11-14.

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-FTIR5, not by normal FTIR. In addition, the detection of peaks by second-order derivative curves 15). 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 16-20).

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 structure 15). 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 carbonates ions at the PO4-site, known as B-type carbonate, in the apatite crystal structure 16-19, 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 analysis 27).

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 portion 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 normal8, 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.

References

1. WHO:Sugars and dental caries. Available at http://www.who.int/iris/handle/10665/259413, 2017
2. Rention C. Managing dental erosion. BDJ Team 2014: doi: 10.1038/bdjteam.2014.109
3. Carvalho TS, Schmid TM, Baumann T and Lussi A. Erosive effect of different dietary substances on deciduous and permanent teeth. Clin Oral Investig 21:1519-1526, 2017
4. Roopa KB, Pathak S, Poornima P and Neena IE. White spot lesions: a literature review. J Paediatr Dent 3: 1-7, 2015
5. Featherstone JD. Dental caries: a dynamic disease process. Aust Dent J 53: 286-291, 2008
6. LeGeros RZ. Chemical and crystallographic events in the caries process. J Dent Res 69 Spec No: 567-574, 1990
7. Yilmaz H and Keleq S. Recent methods for diagnosis of dental caries in dentistry. Meandros Med Dent J 19: 1-8, 2018
8. Sakae T, Nakada H and LeGeros JP. Historical review of biological apatite crystallography. J Hard Tissue Biol 24: 111-122, 2015
9. Sakae T. X-ray diffraction and thermal studies of crystals from the outer and inner layers of human dental enamel. Arch Oral Biol 33: 707-713, 1988
10. LeGeros RZ, Sakae T, Bautista C, Retino M and LeGeros JP. Magnesium and carbonate in enamel and synthetic apatites. Adv Dent Res 10: 225-231, 1996
11. Gotouda H, Nasu I, Kono T, Ootani Y, Kanno T, Tamamura R, Kudawa KT, Suzuki K, Hirayama T, Hirayama T, Sakae T and Okada H. Erosion by an acidic soft drink of human molar teeth assessed by X-ray diffraction analysis. J Hard Tissue Biol 26: 81-86, 2017
12. Rieppo L, Sarakakkala S, Närhi T, Helminen HJ, Jurvelin JS and Rieppo J. Application of second derivative spectroscopy for increasing molecular specificity of Fourier transform infrared spectroscopic imaging of articular cartilage. Osteoarthritis Cartilage 20: 451-459, 2012
13. Zezella DM, Benettia C, Velosoa MN, Castro PAA and Ana PA. FTIR spectroscopy revealing the effects of laser and ionizing radiations on biological hard tissues. J Braz Chem 26: 2571-2582, 2015
14. Cimdina LB and Borodajenko N. Research of calcium phosphates using Fourier transform infrared spectroscopy. In: Infrared Spectroscopy: Materials Science, Engineering and Technology, ed by Theophanides T, InTechOpen Ltd., London, 2012, pp123-148.
15. Drouet C. Apatite Formation: Why It May Not Work as Planned, and How to Conclusively Identify Apatite Compounds. BioMed Research International 2013: doi:10.1155/2013/490946
16. Ando M, Fontana M, Eckert GJ, Arthur RA, Zhang H and Zero DT. Objective and quantitative assessment of caries lesion activity. J Dent 2018: doi: 10.1016/j.jdent.2018.08.009
17. Drouet C, Aufray M, Martinet SR, Vandecandelaère N, Grossin D, Rossignol F, Champion E, Navrotsky A and Rey C. Nanocrystalline apatites: The fundamental role of water. Am Mineral 103: 550-564, 2018
18. Boskey AL and Imbert L. Bone quality changes associated with aging and disease: a review. Ann N Y Acad Sci 1410: 93-106, 2017
19. Paschalis EP, Gamsjaeger S and Klaushofer K. Vibrational spectroscopic techniques to assess bone quality. Osteoporos Int 28: 2275-2291, 2017
20. Henmi A, Okata H, Anada T, Yoshinari M, Mikami Y, Suzuki O and Sasano Y. Bone matrix calcification during embryonic and postembryonic rat calvarial development assessed by SEM-EDX spectroscopy, XRD, and FTIR spectroscopy. J Bone Miner Metab 34: 41-50, 2016
21. Fleet ME. Infrared spectra of carbonate apatites: Evidence for a connection between bone mineral and body fluids. Am Mineral 102: 149-157, 2017
22. Legeros RZ. Effect of carbonate on the lattice parameters of apatite. Nature 206: 403-404, 1965
23. McConnell D. The crystal chemistry of carbonate apatites and their relationship to the composition of calcified tissues. J Dent Res 31: 53-63, 1952
24. McConnell D and Gruner JW. The problem of the carbonate-apatites. III. Carbonate-apatite from Magnet Cove, Arkansas. Am Mineral 25: 157-167, 1940
25. Legeros RZ, Trautz OR, Legeros JP, Klein E and Shirra WP. Apatite crystallites: effects of carbonate on morphology. Science 155: 1409-1411, 1967
26. Hallsworth AS, Weatherell JA and Robinson C. Loss of carbonate during the first stages of enamel caries. Caries Res 7: 345-348, 1973
27. Rey C, Collins B, Goehl T, Dickson IR and Glimcher MJ. The carbonate environment in bone mineral: a resolution-enhanced Fourier transform infrared spectroscopy study. Calcif Tissue Int 45: 157-164, 1989
28. Sakae T. Variations in dental enamel crystallites and microstructure. J Oral Biosci 48: 85-93, 2006
29. Robinson C, Weatherell JA and Hallsworth AS. Distribution of magnesium in mature human enamel. Caries Res 15: 70-77, 1981
30. Weatherell JA, Weidmann SM and Hamm SM. Density patterns in enamel. Caries Res 1: 42-51, 1967
31. Hallsworth AS, Robinson C and Weatherbell JA. Mineral and magnesium distribution within the approximal carious lesion of dental enamel. Caries Res 6: 156-68, 1972