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Abstract: BACKGROUND: Sialolith is a pathological calcification found inside salivary glands, composed principally of apatite crystals. The purpose of this study was to investigate detailed structural properties to gain insight into the formation of sialolith. METHODS: A 45 mm long sialolith from a human submandibular gland was studied using micro-FTIR and three types of X-ray diffraction (XRD) techniques: i) conventional, ii) micro-focus, and iii) "selected-area XRD" (SA-XRD). RESULTS: The longitudinal section of the sialolith showed a stratified structure perpendicular to the long axis. XRD and FTIR showed that the bulk of the sialolith was composed of carbonate hydroxyapatite. Micro-XRD revealed that crystal orientation was slightly different in each incremental zone. "SA-XRD" showed that the average unit cell dimensions of apatite crystals were different for each zone. CONCLUSION: These results suggest that the stratified structure in the sialolith was a result of intermittent and incremental additional growth reflecting compositional changes in the microenvironment.

Key words: Salivary Gland Calculi, Carbonate apatite, FTIR, X-Ray Diffraction

Introduction

Sialoliths are pathological calcifications sometimes occurring in the salivary glands. In our previous paper, we reported a 45 mm long sialolith from a submandibular Wharton’s duct showing a stratified (banded) structure similar to those observed in sialolith from other salivary glands2-5. In general, banded textures in hard tissues indicate that the calcification process occurred by intermittent growth. The banded structure observed in some sialoliths suggest intermittent mineralization. Sialoliths consist of mostly a mineral phase. Like human dental calculus, the mineral phase of sialoliths is usually composed of different calcium phosphates (apatite, whitlockite, octacalcium phosphate), with apatite showing the greatest abundance and occurring in greatest frequency5-7. There is a clinical need for treatment and prevention of sialolithiasis. The purpose of this study is to investigate detailed structural properties of sialolith to gain insight into its formation. For this study, three X-ray diffraction (XRD) analytical techniques, in addition to FTIR, were used to reveal the crystallographic characteristics of the unusually long sialolith: conventional XRD for bulk analysis, micro XRD for point analysis and crystal orientation analysis, and a new technique, “selected-area XRD” for analysis of specific selected areas, having the potential of the data accuracy same to conventional XRD and the spatial resolution of point-analysis similar to that of micro XRD.

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Materials and methods

Salivary calculus used in this study was the unusually long sialolith from the submandibular Wharton’s duct described in\(^1\). It is yellowish-white in color, a slightly bent and wedge-shaped cylindrical form with rounded end, 45 mm x 7 mm in size and 1.34 g in weight. The sialolith was cut longitudinally into halves for X-ray diffraction analysis. The sectioned plane showed a stratified texture normal to the long axis under a binocular scope. The histological and microscopic examination indicated that this sialolith was composed of intermittent layers of calcified materials. To investigate the crystallographic character of the sialolith, three kinds of X-ray diffraction analysis were carried out using the following conditions.

Conventional X-ray Diffraction (XRD)

For this powder-type of XRD analysis, data was collected using a RIGAKU RINT 2000, powder-type X-ray diffractometer, RIGAKU Co. Ltd. Tokyo, under the following conditions: X-ray generation: Cu rotary target, accelerating voltage: 50 kV, accelerating current: 200 mA, monochromator: graphite plate crystal, X-ray wavelength: 1.5418 Angstrom (CuK\(_\alpha\)), detector: scintillation counter, scan speed: 1 degree (2θ/min), scan range: 3-120 degree (2θ). X-ray data analysis was carried out using JADE (Material Data Inc.), using conventional slit-system. The X-ray radiation area on the sample was 10mm x 20mm at the low angle region.

Micro X-ray Diffraction (Micro-XRD)

Micro-focus X-ray diffraction (micro-XRD) data of the sialolith from a point 100 μm in diameter was collected using a RIGAKU RINT RAPID with IP (imaging plate) system, RIGAKU Co. Ltd. Tokyo. The image data was digitally recorded and directly transferred to a computer. The experimental conditions were as follows; X-ray tube target: Cu, tube voltage: 50 kV, tube current: 30 mA, monochromator: graphite plate crystal, X-ray wavelength: 1.5418 Angstrom (CuK\(_\alpha\)), collimator: 100 μm in diameter, Imaging Plate® (IP, Fuji Film Co. Ltd., Tokyo) recording system with IP size: 466 mm x 256 mm, camera radius: 127.4mm, pixel size: 100 μm x 100 μm, sample setting: reflection-mode, exposure time: 20 min. X-ray diffraction IP imaging analysis system: RIGAKU R-AXIS display software.

Selected-Area X-ray Diffraction (SA-XRD)

In this study, another type of micro-XRD analysis was applied. The auto-slit-width-arrange-system, equipped on the RIGAKU RINT 2000, allows a fixed X-ray exposed area on a sample plane at any given scanning angle by controlling the X-ray incident beam width. In this study, the X-ray exposed width was fixed at 1 mm on the sample plane, and then the radiation window area was 1mm width x 10mm length. The other experimental conditions were similar to the conventional XRD measurement. This method that we refer to as “Selected-Area X-ray diffraction” (SA-XRD), has not been used in the investigation of pathological materials. This method has some advantages in data-collection time, accuracy of data and data compatibility with conventional powder XRD method compared with the micro-focus XRD. Applying this method to the specimen, the window area of 1 mm in width was set perpendicular to the longitudinal axis of the sialolith. The unit cell dimensions were calculated using the JADE program.

Micro Fourier Transform Infrared Analysis (Micro FT-IR)

Carbonate and phosphate composition was analyzed using a micro FT-IR, HORIBA FT-530, under the following conditions; measurement mode: reflection, analyzing area: 20x100 μm, detector: MCT (cooling by LN\(_2\)), Cassegrain mirror: x4, measurement cycle: 60, resolution: 4cm\(^{-1}\), measurement range: 750-4000 cm\(^{-1}\). The measurements were carried out at the positions where the XRD studies were carried out. The observed raw data was transformed to a pseudo-transmitting absorption spectrum by the Kramers-Krönig analysis.

Results

The long sialolith was divided into three portions namely as the thin head-portion, the body-portion at the middle and the thick tail-portion, for placing the sample in the sample holder (Fig. 1). The sialolith showed a stratified texture on the sectioned plane with a loose concentric pattern. These bands consisted of alternating narrow and wide bands. The wide bands were relatively glossy and transparent, suggesting that relatively large crystals are arranged in an organized manner in contrast with the crystals located in the narrow slightly brownish bands.

The conventional-type XRD patterns of the three portions showed apatite structure (Fig. 2). The conventional XRD patterns showed the diffraction peak broadening which was usually observed in biologic apatites. No other crystalline phase was detected in these patterns.

Micro-XRD IP images of thirty points on the sections of the three portions were obtained. An example of a micro-XRD pattern is shown in Fig. 3. The two-dimensional micro-XRD image clearly showed preferred crystal orientation as the strong 002 circular arc. A series of micro-XRD images of the sialolith showed that there were slight differences in the preferred crystal orientation among the neighboring points. The converted powder pattern of this micro-XRD data (Fig. 4) identified the crystals as apatite.

All the sixty SA-XRD patterns of the sialolith were identified as apatite (Fig. 5). There were small differences in the peak positions among the analyzed areas, indicating possible differences in composition of the apatite. The unit cell dimensions of the apatite crystals are summarized in Table 1. These values were slightly, but significantly, different from each other. The variations in the unit cell dimensions indicated that the crystals in incremental bands slightly differed in chemical composition resulting from changes in the type and amount of substituting ions in the apatite crystal.
Fig. 1. Photograph of the head-portion section plane of the unusually long sialolith, showing the selected area analysis points at the cross-points of the lines. The tangential axis line also indicated the line of points for the micro XRD analysis.

Fig. 2. Conventional XRD pattern of the head-portion section, showing an apatitic diffraction pattern.

Fig. 3. Micro-XRD IP image from a 100 μm in diameter point on the section of the sialolith. The right portion of the image was hidden by sample holder. The 002 reflection (arrow) showed arcs in stead of the complete diffraction ring, indicating preferred crystal orientation.

Fig. 4. Converted XRD pattern of the micro-XRD image (Fig. 3) showing the apatite diffraction peaks.

Fig. 5. SA-XRD pattern of the sialolith showing apatite diffraction peaks.

Fig. 6. Micro FT-IR spectrum of the sialolith showing the absorption bands of phosphate ions at about 1030 cm⁻¹ and the bands of carbonate ions at about 1450 cm⁻¹ and 1550 cm⁻¹.

lattice.

The micro FT-IR analysis revealed the main constituent of the sialolith was carbonate apatite, carbonate absorption bands appearing at about 1400 cm⁻¹ and 1600 cm⁻¹ (Fig. 6).

Discussion
In this study, three types of XRD were applied to reveal the crystallographic characters of the sialolith. The conventional XRD
Table 1. Crystallographic values (unit cell dimensions) of apatite crystals from different areas of the sialolith analyzed using selected-area XRD.

<table>
<thead>
<tr>
<th>position No.</th>
<th>a-axis length* (Å)</th>
<th>b-axis length* (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head 01-1</td>
<td>9.412(11)</td>
<td>6.893(12)</td>
</tr>
<tr>
<td>Head 02-1</td>
<td>9.433(16)</td>
<td>6.888(18)</td>
</tr>
<tr>
<td>Head 03-1</td>
<td>9.426(25)</td>
<td>6.889(21)</td>
</tr>
<tr>
<td>Tail 01-1</td>
<td>9.405(23)</td>
<td>6.903(26)</td>
</tr>
<tr>
<td>Tail 01-2</td>
<td>9.430(15)</td>
<td>6.900(12)</td>
</tr>
<tr>
<td>Tail 01-3</td>
<td>9.440(22)</td>
<td>6.871(19)</td>
</tr>
<tr>
<td>Tail 02-1</td>
<td>9.421(14)</td>
<td>6.886(11)</td>
</tr>
<tr>
<td>Tail 03-1</td>
<td>9.431(18)</td>
<td>6.880(13)</td>
</tr>
<tr>
<td>Tail 04-1</td>
<td>9.423(10)</td>
<td>6.874(08)</td>
</tr>
</tbody>
</table>

*: The estimated standard deviations were represented in parenthesis.

showed the sialolith being composed of apatite. The micro-XRD showed the preferred crystal orientation in the sialolith. SA-XRD analysis, used for the first time in the analysis of pathological calcifications revealed inhomogeneity in the chemical composition of apatite from layer to layer. The intermittent growth of the apatite crystals was confirmed by crystal orientation analysis.

X-ray diffraction analysis provided information on the crystalline phases present in the sialolith. However, in the present study, the diffraction peaks of the apatite crystals in the sialolith were too broad to allow accurate analysis. Variations in the unit cell dimensions strongly suggested changes in apatite composition, reflecting changes in the environmental condition and composition.

Information on the crystalline phase, composition and orientation, could lead to a better understanding of the formation mechanism of sialolith and may provide insights on the appropriate treatment regimen for lithotripsy.

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References