

# Micromorphology and Chemical Composition of a Sialolith in the Submandibular Gland Duct

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Sialolith is one of the most common pathologic conditions found in the salivary glands. The mechanisms responsible for the formation of sialoliths have not been elucidated so far. In this article, the chemical composition and micromorphology of a sialolith of a 58-year old female patient suffering from chronic sialoadenitis of the submandibular gland was analyzed using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). In a SEM evaluation, the highly mineralized amorphous core surrounded by lamellar and concentric structures was revealed, however no foreign body, organic material, or signs of microorganism were observed in the core of the sialolith. EDX analysis showed the central core was composed of only Ca, O and P, and that a high level of C was detected near the central area as well. These results indicated that the inorganic composition of the sialolith was hydroxyapatite crystals, and that inorganic and organic substances existed around the central cores. This study suggests that the sialolith was composed mainly of hydroxyapatite crystals and the formation of the nucleus of the sialolith in the submandibular gland duct was secondary to sialadenitis, which favors the growth of an inorganic crystalline nucleus.

**Key words :** Sialolith, Hydroxyapatite, Sialadenitis, Scanning electron microscopy, Energy-dispersive X-ray spectroscopy

## I. INTRODUCTION

Sialolith is one of the most common pathologic conditions found in the salivary glands. Obstruction of the salivary gland by a sialolith is associated with pain and inflammation, and in some cases with an

infection of the affected gland.<sup>1)</sup> The submandibular gland is more frequently involved (83% to 94%) than are the parotid (4% to 10%) or sublingual glands (1% to 7%), probably because of its more viscous saliva, longer duct, and higher mineral content in the saliva.<sup>1)</sup> This disease condition is more frequent in adults (0.1 - 1.0% of population) than in children.<sup>2-3)</sup> In general, sialoliths are composed of an organic and inorganic matrix, and have a central core and laminar peripheral structures. A major component of sialoliths is calcium phosphate.<sup>4-6)</sup> Many theories have been suggested to explain sialolith formation, including calcification around foreign bodies, desquamated epithelial cells, and microorganisms in the duct.<sup>1,7)</sup> However, the formation of the crystals in human salivary glands and their role in the

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pathogenesis of the sialolithiasis has not yet been elucidated.

Therefore, the aim of this study was to analyze a sialolith using a SEM and EDX to conduct a chemical analysis on the sialolith and to determine the presence of a foreign body or organic materials related with the formation of a nucleus in the center of the sialolith.

## II. METHODS

A 58-year old female patient visited the clinic complaining of the symptoms of pain and swelling in her mouth floor and submandibular region associated with chronic sialadenitis. The sialolith was excreted from her left submandibular gland duct spontaneously 3 weeks after treatment with antibiotic and analgesic medication. Her symptoms almost improved after excretion of the sialolith. The discharged sialolith had an oval shape with narrow tips and was 5 mm in length (Fig. 1).

The sialolith was rinsed in distilled water and air dried. The sialolith sample was fractured and divided longitudinally into two halves. One half was prepared for scanning electron microscopic (SEM) evaluation and energy-dispersive X-ray spectroscopy (EDX). SEM and EDX examination was carried out using a scanning electron microscope (S-3000N, Hitachi, Japan) with an attached energy-



Fig. 1. Clinical photo of the sialolith spontaneously excreted from the submandibular gland duct of a patient suffering from chronic sialadenitis.

dispersive X-ray microanalysis detector system (EMAX ENERGY, Horiba Ltd., Japan). The sialolith piece kept for SEM was coated with a thin film of evaporated Au-Pd in order to avoid electric charge on the surface. The SEM study and EDX microanalysis were performed at an accelerating voltage of 15.0 kV and a working distance of 16 mm. After calibration setting, several representative micrographs were obtained from SEM. The elemental distribution and atomic weights were measured by EDX.

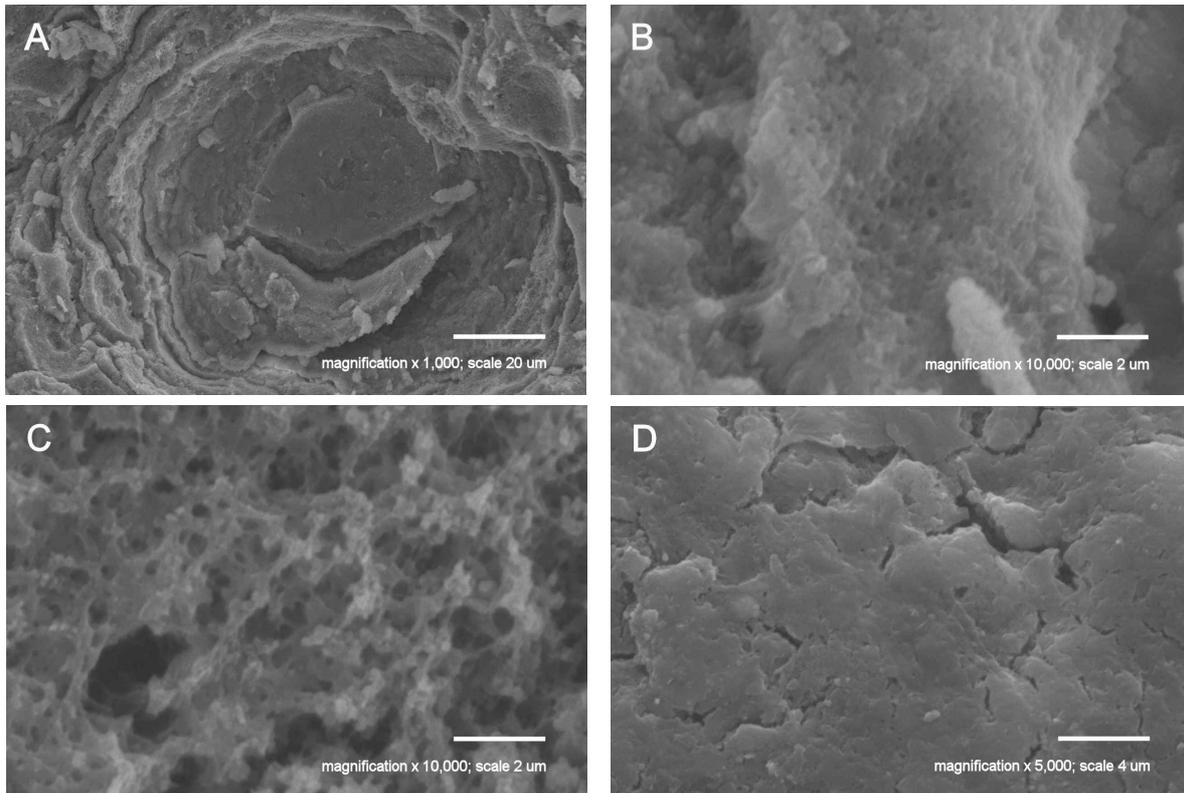
## III. RESULTS

SEM images of the sialolith depicted the highly mineralized amorphous core surrounded by lamellar and concentric structures. There was no foreign body or organic materials in the center of the sialolith (Fig. 2A). Main elements determined by the EDX analysis on the central area of the fracture surface were Ca, P and O (Table 1).

Around this amorphous nucleus, organic and inorganic substances accumulated in laminar layers. Microcrystalline structures were identified in an outer shell area near the central core of the sialolith (Fig. 2B). As well as the main elements of Ca, P and O, a relatively high proportion of C was detected some distance from the central area, indicating the abundance of organic materials (Table 2).

Another outer shell area was extremely porous, which suggests the possibility of a composite formation mechanism from organic and inorganic materials (Fig. 2C). The organic material was predominantly concentrated in the outer shell of the sialolith, however structures corresponding to a foreign body or bacteria were not recognized anywhere.

The external surface of the sialolith was generally smooth but had numerous irregular cracks and crevices (Fig. 2D). Other elements such as small proportions of Na, Mg, Al, Si, and S were detected on the external surface matrices of the sialolith (Table 3). Nonetheless, dominant presence of Ca, P and O at all points analyzed with EDX highly suggested the existence of hydroxyapatite.



**Fig. 2.** Scanning electron microscopic images of the sialolith.

- A. A central core area of the sialolith piece showing a concentric laminar structure without a foreign body or microorganisms related with the formation of a nucleus.
- B. An outer shell area near the central core of the sialolith showing microcrystalline structures.
- C. Another outer shell area of the sialolith having an extremely porous structure, suggesting the composite formation from organic and inorganic materials.
- D. An external surface area of the sialolith exhibiting smooth surface with irregular cracks and crevices.

**Table 1.** The EDX analysis of the central core area of the fractured surface shown in Figure 2A: The atomic percentages (at %) and the weight percentages (wt %) of the elements were determined by the EDX analysis of the sialolith piece.

Element	wt %	at %
O	57.51	75.44
P	15.07	10.21
Ca	27.42	14.36

**Table 2.** The EDX analysis of the outer shell area near the central core of the fractured surface shown in Figure 2B.

Element	wt %	at %
C	22.25	36.59
O	32.50	40.12
P	6.84	4.36
Ca	38.41	18.93

**Table 3.** The EDX analysis of an area of the external surface of the sialolith shown in Figure 2D.

Element	wt %	at %
O	64.24	79.23
Na	1.05	0.90
Mg	1.22	0.99
Al	1.00	0.73
Si	1.37	0.97
P	11.71	7.46
S	2.13	1.31
Ca	16.08	7.92
Ti	1.18	0.49

#### IV. DISCUSSION

This article investigated the chemical composition and micromorphology of a sialolith using energy-dispersive X-ray spectroscopy and scanning electron microscopy. In a SEM evaluation, the highly mineralized amorphous core surrounded by lamellar and concentric structures was revealed, but no foreign body, organic material, or signs of microorganism were found in the core of the sialolith.

In general, sialoliths are composed of an organic and inorganic matrix, and present highly mineralized cores surrounded by concentric alternating mineralized and organic layers.<sup>4-6,8-9)</sup>

Specific structures and patterns are also reported such as numerous microstructures of different shapes (nodular, laminar, reticular, microgranular, and multinodular) and sizes arranged in a haphazard fashion<sup>10)</sup> and irregular, partly rudely hexagonal, needle-like and plate-shaped crystals.<sup>11)</sup> Granular or globular and pyramid structures were found on the surface of the sialolith.<sup>12)</sup> Significant variability could be identified among the specimens. Some sialoliths exhibited multiple cores and lacked concentric laminated structures and other specimens disclosed extensive regions of globular structures.<sup>9)</sup>

Previous SEM studies on sialoliths have described the development of a core formation from organic matter and microorganisms. Microorganisms have been found in the intermediate and peripheral parts of the calculi, but not in the nucleus.<sup>6,13)</sup> In other SEM studies, a scaly structure corresponding to fiber and bacteria was recognized,<sup>12)</sup> and even filamentous and rod-shaped bacteria were demonstrated in the outer layer of sialoliths.<sup>7)</sup> On the contrary, this study failed to detect any evidence of a foreign body, organic material, or microorganisms from the sialolith, and this is consistent with a study by Kasaboglu<sup>11)</sup> reporting similar findings.

In this study, EDX analysis showed the central core contained only Ca, O and P, and that a high level of C was detected near the central area as well. These findings indicated that the principal inorganic composition of the sialolith was hydroxyapatite crystal, and that inorganic and organic substances existed around the central cores.

Many studies have shown that the main elements of sialoliths are Ca and P.<sup>11-15)</sup> In a study using Fourier transform infrared, FT-Raman, and fluorescence spectroscopic techniques, the ratio of the major elements of a sialolith was calculated and it was 7:3 for Ca and P.<sup>16)</sup> In addition to these primary elements, sialoliths often contain small amounts of Mg, Na, Cl, Si, Al, Fe, and K.<sup>11,13-15)</sup> The amounts of Na, Mg, Cl, Si, Fe, and K are higher in external and internal surfaces than in the central parts of sialoliths.<sup>11)</sup> Studies on crystalloids in salivary duct cysts and in the minor salivary gland have shown that sulfur compounds were one of the main components of the crystalloids.<sup>14-15,17)</sup>

Previous studies on submandibular sialoliths revealed that the inorganic components include apatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , whitlockite  $\text{Ca}_3(\text{PO}_4)_2$  and brushite  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ .<sup>18-19)</sup> Among these components, hydroxyapatite is the main component of sialolith crystals in both inner and outer parts.<sup>11,14,19-20)</sup> Sialoliths generally consist of mixtures of different calcium phosphates (mainly hydroxyapatite and carbonate-apatite) together with an organic matrix.<sup>3,6,20-21)</sup> The nucleus of the sialoliths is mostly

inorganic, predominantly made up of calcium phosphate and calcium carbonate in the apatite structure. Around this amorphous nucleus, laminar layers of organic and inorganic substances accumulate, and their content varies within a single sialolith.<sup>22)</sup> The organic material is predominantly concentrated in the outer shell of the sialoliths and their components are mostly glycoproteins, mucopolysaccharides, lipids and cell detritus.<sup>22)</sup> A study using Fourier transform infrared, FT-Raman, and fluorescence spectroscopic study has proven that the organic components of a sialolith are structural proteins and amino acids.<sup>16)</sup> Interestingly, there are sialoliths exclusively constituted by organic materials.<sup>8)</sup>

The findings of this study suggest the sialolith was composed mainly of hydroxyapatite crystals and the formation of the nucleus of sialolith in the submandibular gland duct was secondary to sialadenitis, which favors the growth of inorganic crystalline nucleus.

Many theories have been stipulated to explain salivary calculi formation, such as calcification around foreign bodies, desquamated epithelial cells, and microorganisms in the duct.<sup>1,7)</sup> According to Hiraide<sup>13)</sup>, a foreign body or microorganism acts as a nucleus of initial stone formation and the nucleus might be morphologically changed during the calculus development so that the foreign body or microorganism eventually loses its contour. Takeda<sup>17)</sup> proposed a different mechanism with which the crystalloids present in the parotid gland may aggregate to form nuclei of calculi on which successive layers of organic and inorganic material from the saliva would be deposited. Harrison<sup>23)</sup> explained that the formation of the nucleus of sialoliths in the submandibular glands is secondary to sialadenitis. During chronic submandibular sialadenitis, inflammatory swellings lead to the partial obstruction of a large duct with stagnation of secretory material rich in calcium. This would form a calcified core and later, a sialolith.

Differences in the structure and mineralization of sialoliths indicate that their formation is influenced

by several factors. One factor is saliva retention due to morphoanatomic factors including salivary duct stenosis and salivary duct diverticuli.<sup>24)</sup> Salivary composition factors were presented, such as high supersaturation and crystallization inhibitors deficit.<sup>25)</sup> Deficit of crystallization inhibitors such as myo-inositol hexaphosphate (phytate) was suggested as an important etiologic factor implied in the sialolith development.<sup>8)</sup> Formation of sialoliths is promoted by abundant organic substances, in particular of glycoproteins with high calcium affinity, accumulation of calcium and phosphate, and alterations in pH.<sup>22)</sup> Further research is needed to establish the exact mechanisms responsible for the formation of sialoliths.

## V. CONCLUSION

The chemical composition and micromorphology of a sialolith of a 58-year old female patient suffering from chronic sialoadenitis of the submandibular gland was analyzed using a SEM and EDX. This study suggests that the sialolith was composed mainly of hydroxyapatite crystals. Furthermore, it implies the formation of the nucleus of the sialolith in the submandibular gland duct was secondary to sialadenitis, which favors the growth of an inorganic crystalline nucleus.

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국문초록

## 악하선 내 타석의 미세형상 및 화학적 조성

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타석은 타액선에 발견되는 흔한 병리 상태 중 하나이다. 타석의 형성과 관련된 기전은 아직까지 명확하게 밝혀지지 않았다. 본 연구에서는 악하선의 만성타액선염을 앓고 있는 58세 여성 환자로부터 얻은 타석을 전자현미경 및 에너지분산 X선 분광법을 통해 화학적인 조성과 미세형상을 분석하였다. 전자현미경 평가에서 고도로 광화된 무정형 핵이 층판의 중심원상 구조로 둘러싸인 형태가 관찰되었으나, 이물질, 유기물질 또는 미생물의 소견은 확인되지 않았다. 에너지분산 X선 분광 분석에서 중심부 핵은 Ca, O 및 P로만 구성되어 있었고, C도 중심부 근처에서 높은 함량으로 검출되었다. 이러한 결과는 타석의 무기물 조성은 주로 수산화인회석 결정으로 되어 있고 중심핵 주변으로 무기-유기 구조가 형성되어 있어, 악하선관 내 타석의 핵이 무기결정핵의 성장을 촉진하는 타액선염에 의해 이차적으로 형성되었을 가능성을 제시한다.

**주제어** : 타석, 수산화인회석, 타액선염, 주사전자현미경, 에너지분산형 X-선 분석

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