Chapter 9

Characterisation of a Submandibular Sialolith Resected from the Wharton's Duct

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9.1 Introduction

Sialoliths are calcified organic matter that develop in the parenchyma or ducts of major or minor salivary glands. The crystalline structure is chiefly hydroxy apatite and contains octacalcium phosphate. The composition appears laminated with layers of organic material covered with concentric shells of calcified matter (1). Because the type of stone, based on its major component, is related to its pathogenesis, the identification of the stone is a prerequisite in the diagnosis and prevention of recurrence. Vibrational spectroscopic study can help the molecular characterisation of proteins and keratotic bio-polymers of complex human biological samples such as hair, callus, skin and cancerous tissues (2,3,4). Several studies have been reported on the application of infrared and Raman spectroscopic techniques to study the composition of human teeth (5,6). Moreover, these techniques have been proved to be the ideal methods for the diagnosis and identification of stones that are broadly classified into gall stones in the gall bladder and kidney stones in the urinary tract (7). However, little report is available on the vibrational spectroscopic studies of stones in the salivary glands or sialoliths.

In this chapter, the sialolith resected from a male patient who had complaint of severe pain and swelling of the right angle of mouth on taking food for one month is studied using vibrational spectroscopic
analysis and other characterisation methods like thermal analysis and Energy Dispersive Analysis of X-ray data.

9.2 Materials and Methods

The sialolith used in this study was resected from the Wharton’s duct using a 15W Nd: YAG laser used in the continuous mode. The resected sample was cut into small parts for various studies. FTIR spectrum of the sample in the 400-4000 cm⁻¹ was recorded using a Nicolet Inc. IMPACT 410 FTIR spectrophotometer (Madison, USA) with the sample taken as KBr pellets. FT-Raman spectrum of the powdered sample of the sialolith in the 400-4000 cm⁻¹ region was recorded using IFS-66 Bruker spectrometer connected with a Raman module (Bruker, FRA-106) with radiation from a Nd: YAG laser of about 200 mW at the sample. SEM – S2400 (Hitachi) model was used to get the SEM of the sample. The thermal analysis was carried out in the 303-573K and 573-1673K regions using a TA instrument (Model SDT 2960 simultaneous DTA – TGA). EDAX was studied using a Link ISIS Oxford Instruments with a system resolution of 193 ev.

9.3 Results and Observations

The FT-Raman and FTIR spectra of the sialolith are given in Figs 9.1 and 9.3 respectively. The FT–Raman spectrum obtained in the present study is similar to the spectrum of the dentine part of modern
tooth reported by Kirchner and Edwards (Fig.9.2) (8). Assignments of the bands in the present study are carried out in comparison with the assignments of dentine material and those of the crystalline structure of sialoliths (1,9). Results of Energy Dispersive Analysis of X-ray Diffraction study undertaken in the present investigation are also made use of. The observed bands are assigned on the basis of the vibrations of the inorganic hydroxy apatite component and the organic component. A gross photograph of the sialolith immediately after resection from the duct is shown in Fig 9.6.

9.3.1 Inorganic Component

9.3.1.1 $PO_4^{3-}$ Vibrations

The most intense band observed at 962 cm$^{-1}$ is assigned to the Raman active symmetric stretching mode of phosphate group which is characteristic of the inorganic hydroxy apatite component. A very weak band at 960 cm$^{-1}$ is observed in the IR spectrum for this mode. The asymmetric stretching mode is observed as weak bands at 1045 cm$^{-1}$ and 1070 cm$^{-1}$ in the Raman spectrum. This IR active mode appears as a very intense band at 1031 cm$^{-1}$ with a shoulder at 1095 cm$^{-1}$. The symmetric and asymmetric bending modes of the $PO_4$ group are also observed as two bands each in the IR and Raman spectra in the 430-485 cm$^{-1}$ and 564-615 cm$^{-1}$ regions.
The three fundamental vibrations connected with the hydrogen bonded OH groups are $\nu$(OH) stretching, in-plane $\delta$(OH) and out-of-plane $\gamma$(OH) bending vibrations. In a strongly hydrogen bonded system, $\nu$(OH) band is usually very broad due to strong interactions between proton vibrations and $\nu$(O…O) vibrations (10,11). The overtones of the $\delta$(OH) and $\gamma$(OH) modes in Fermi resonance with the broad $\nu$(OH) band cause the appearance of three bands A, B and C in the $\nu$(OH) stretching region (12).

The very broad and intense band observed at 3440 cm$^{-1}$ in the IR spectrum corresponds to the stretching mode of bound water molecules contained in the sample. The presence of bound water molecules in the sample is confirmed from the results of DSC study conducted in the 303-573K region which shows a sharp curve at 371.57K due to the loss of weight of water molecules (Fig 9.4). The broad band extending from 2500–3750 cm$^{-1}$ in the FTIR spectrum also includes the stretching vibrations of N-H modes of the amide group. In the Raman spectrum a very weak band at 3285 cm$^{-1}$ is assigned to the stretching mode of the H$_2$O molecule. The bending mode of water cannot be distinguished as the amide bands appear in the same region. The bands observed at 2855 cm$^{-1}$ and 1925 cm$^{-1}$ in IR are assigned respectively to the A and B bands. The C band is not identified as it is masked by the Amide I bands. The
appearance of A and B bands indicates the presence of strong hydrogen bonds in the sample. Eventhough the Raman spectrum of the dentine material and sialolith appears to be similar, the bands due to OH vibrations are not assigned in Kirchner’s work (8).

9.3.2 Organic Component

Proteinaceous component is identified as the chief constituent of the organic part of dentine material. The vibrational bands at 2940, 1685, 1667, 1451 and 1270 cm\(^{-1}\) are characteristic of this component (8). Bands are obtained in the same region in the present study indicating that the organic part corresponds to that of proteinaceous component. The bands of sialoliths corresponding to organic part are assigned in comparison with those of the protein contents of the biopolymers and collagen spectral assignments (13, 14).

The medium intense bands observed at 1660 and 1647 cm\(^{-1}\) in the IR spectrum and the weak bands at 1664 and 1650 cm\(^{-1}\) in the Raman spectrum are assigned to the amide I bands which corresponds to the carbonyl stretching vibration (80%). This agrees with the assignments of the amide I band of bovine Achilles tendon (14). Two amide I bands have also been identified in the Raman spectrum of dentine material (8). The weak bands observed at 1570 cm\(^{-1}\) in the IR and 1595 cm\(^{-1}\) in the Raman spectrum corresponds to the amide II mode which predominantly
is the sum of N-H deformation vibration and C-N stretching vibration.
The bands of medium intensity at 1458 cm\(^{-1}\) in the IR and 1448 cm\(^{-1}\) in
the Raman are characteristics of \(\delta(CH_3)\) and \(\delta(CH_2)\) modes. The amide
III band is observed at 1311 cm\(^{-1}\) in the Raman spectrum. In \(\alpha\)-helical
structures, the amide I mode occurs as an intense band in the Raman
spectra around 1652 cm\(^{-1}\) (15). The amide I band in the \(\beta\)-sheet structure
can range from 1658-1679 cm\(^{-1}\) region (14,16). Hence the bands
observed between 1647 cm\(^{-1}\) in the IR spectrum and at 1650cm\(^{-1}\) in the
Raman spectrum are assigned to the Amide I mode of the \(\alpha\)-helical
structure and the bands at 1660 and 1664 cm\(^{-1}\) are assigned to the amide
I mode of the \(\beta\)-sheet conformation. Chen and Lord have summarised
the amide III frequencies of various conformations and have assigned a
medium band at 1265 – 1300 cm\(^{-1}\) for the \(\alpha\) – helix conformation (17).
Hence, the band appearing at 1311 cm\(^{-1}\) in the present study is assigned
to the \(\alpha\) - helical conformation of the amide III mode. The amide II band
comprising the \(\nu(C-N)\) stretching vibrations, which is usually associated
with \(\alpha\)-helical conformation, is also observed as a weak band in the
Raman spectrum (Table 9.1). The amide III band of the \(\beta\) -sheet
conformation, which is expected in the 1270-1220 cm\(^{-1}\), is not observed
in this study. The well defined but weak band at 1004 cm\(^{-1}\) is assigned
to the aromatic ring stretching vibration of phenylalanine units which is
one of the distinctive side chains in collagen spectrum. The vibration of
yet another side chain hydroxy proline is observed as a weak band at 875 cm$^{-1}$ in the IR spectrum. The N-H stretching vibrations are expected around 3350 cm$^{-1}$ for the bonded primary amides. This band is merged with the broad water band and appears around 3440 cm$^{-1}$ as a very intense broad band. The stretching mode of CH$_3$ is observed as weak band at 2927 cm$^{-1}$ in the IR spectrum. The medium intense band observed at 2897 and 2934 cm$^{-1}$ in the Raman spectrum are assigned to the stretching modes of CH$_2$ and CH$_3$ respectively.

9.3.3 Thermal Studies, Microstructure (SEM) and Trace Elements Analysis

Thermal decomposition of the material was carried out at a heating rate of 5$°$C/min. The thermogram obtained in the 303-1673K region is shown in Fig 9.4. One endothermic curve at 345.09K and an intense exothermic peak at 660.19K are obtained in the DTA study (Fig 9.5). No phase change or decomposition of constituent molecules is observed in the 303 – 573K ranges. The weight loss when heated to 439K is found to be 7.68%. This may be due to the loss of water and weakly bonded organic components. When temperature is raised to 561 K, 9.53% loss of weight is noted. Even at 1554K, a weight loss of only 32.82% is observed indicating that the inorganic part of the sample is stable without disintegration at this temperature.
The Scanning Electron Micrographs (SEM) of the sample from three different surfaces are shown in Fig. 9.7 to 9.9. The Energy Dispersive Analysis of X-ray diffraction spectrum (Fig 9.10) recorded to identify the trace elements present in the sample shows the presence of calcium and phosphorous in the ratio 7:3.

9.4 Discussions

The IR and Raman spectra of human tooth enamel and dentine have been extensively investigated to study the potential of preventing caries formation and to improve oral hygiene. These studies show that the enamel layer consists of 96.5% inorganic material and they show apatite peaks at 591, 961 and 1071 cm\(^{-1}\) (18). The bands obtained in the Raman spectrum of sialolith are found to be similar to those obtained in the Raman spectrum of dentine material of human teeth (8). Therefore, it can be concluded that the stones formed in the sub mandibular Wharton’s duct are dentine materials. The bands at 960, 1031, 564 and 603 cm\(^{-1}\) of the IR spectrum corresponds to the apatite bands and the bands at 1458, 1417 and 875 cm\(^{-1}\) are due to inorganic minerals. The observed bands of the CH\(_2\) and CH\(_3\) groups confirm the presence of proline and hydroxyproline. Eventhough the etiology of sialolith formation is still defendable the main reasons attributed include sharp curves of Wharton’s duct, high level of calcium in saliva and the dependant position of submandibular gland, which increases the chance
for stasis. It is also reported that disorders of calcium and phosphorous metabolism leads to sialoliths. In this context, the identification of various components of the sialolith may be useful for the medical community to control recurrence of sialoliths. (7)

No changes are observed in the thermal parameters of the sample in temperature range upto 1673K. Hence in-situ laser disintegration of the sialolith by non-invasive method cannot be performed because the use of lasers generates high temperatures increasing the chances of burning the surrounding tissue. Moreover, lithotripsy for the disintegration of sialoliths is reported to be complicated as it causes the destruction of amalgam restorations (19).

9.5 Conclusion

From the vibrational bands observed for the sialolith, the structure of the sialolith is identified to be similar to that of the dentine material. Since it is detected that calcium and phosphorous constitutes the major proportion of the sialolith, reduced consumption of food/drug which are rich in these two elements can prevent the recurrence of sialolith formation. From the results of the DTA/TG analysis, it is evident that an in situ disintegration of the silolith with laser is not possible.