Infra-red and Raman Spectroscopic Studies of Infected and Affected Dentine

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ABSTRACT: *Diagnosis of infected and affected dentine is an important factor in clininal restorative treatment of dentine. In this study ,the IR and Raman spectra of the sound dentine , infected dentine and affected dentine are reported. The structure of infected dentine and affected dentine has been compared with the sound dentine and hydroxyapatite. It is shown while the infected dentine has lost its structure, the affected dentine has more or less a structure similar to the sound dentine. The molecular structure of collagen remains unchanged in both infected and affected dentine.*

KEYWORDS: *IR Spectroscopy, Raman Spectroscopy, dentine, Infected dentine, Affected Dentine, Dental treatment.*

INTRODUCTION

Dentine is a complex bioinorganic material which makes up the bulk hard component of the tooth. Other hard parts of the tooth include enamel which covers the exposed crown of the tooth as a thin protective layer, and cementum which surroundes the dentine at the root.

The composition of human dentine is approximately %75 inorganic, %20 organic material and %5 water. The main part of inorganic component of dentine is carbonated hydroxyapatite which is represented by the formula $Ca_{10} (PO_4)_{6-x} (OH)_{2-y} (CO3)_{x+y}$, where $o \leq x \leq 6$ and $o \leq y \leq 2$. In the apatite structure of dentine a trace amount of ions such as F^-, Na^+, Ba^{2+}, Mn^{2+}, Li^+, Sr^{2+}, Al^{3+}, Mg^{2+}, Cd^{2+} and Pb^{2+} have been identified [1].

The main organic component of dentine is a protein called collagen which serves as a supporting matrix. Other proteins and lipids in minor amounts are also present in dentine [2].

Dental caries , commonly called tooth decay , is the most common chronic disease in the world. This is an infectious microbiological of the teeth which results in dissolution , creating a cavity and finally destruction of the tooth.The dental caries starts from the formation of bacterial plaque on the enamel surface , destruction of enamel and finally progression in dentine.

Dentine caries has been classified as infected dentine and affected dentine [3]. Infected dentine is the outer layer and is soffened and contaminated with bacteria. It is irreversibly denatured and not remineralized . In other hand , affected dentine has a demineralized phase , but not yet invaded by bacteria.

In clinical restorative treatment of dentine during cavity preparation it is infected dentine which is completly removed. The affected dentine , which may be remineralized after the complition of restorative treatment , is not

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removed and is preserved. In clinical procedures to distinguish between these two layers, and for the removal of infected dentine , the operator normaly observes the degree of discoloration and test hardness of the area by the feel of an explorer tine or a slowly revolving bur. This criteria to diagnose dentine which is based on colour and hardness , however , has not a sufficient degree of accuracy and fails to be of a definite indication of the depth and degree of infection. The diagnosis of infected and affected dentine remains therefore a challenge to the science of dentistry.

There have been several investigations on the structure and chemical composition of infected and affected dentine using different techniques [4-6]. Daculsi et al using TEM technique have reported that in infected dentine the dissolution of apatite structure of dentine is accompanied with the reprecipitation of Mg-substituted - TCP (Beta TriCalcium Phosphate) [6]. Kuboki etal have reported the collagen biochemistry of the two layers of carious dentine using chromatographic techniqe [5].

Although there are several IR and Raman spectroscopic studies of dentine and enamel of human tooth [7,8], but as far as it is known , the present work is the frist IR and Raman spectroscopic investigation of infected and affected dentine.

MATERIALS AND METHODS

Materials

Human permanent teeth were collected , washed free of adherent blood products and stored in %0.2 thymol solution. Sound dentine , infected dentine and affected dentine were prepared using a revolving bur.Hydroxyapatite (HAP) was obtained from Aldrich Chemical (Milwaukee , WI) and was used without purification.

Instrumentation

Infrared spectra were recorded using Shimadzu model IR - 435 spectromtre.

Samples of sound dentine , infected dentine and affected dentine were ground in an agate mortar and about 1mg was mixed with 200 mg of ground spectroscopic KBr. Transparent pellets were prepared in a KBr die with an applied load of 7000Kg.

FT-Raman spectra were recorded using a Bomem Model NB-Series equiped with a Te-cooled indium - gallium - arsenide detector. Excitation wavelength at 1064 nm was obtained from a Nd/YAG laser. Samples of ground sound dentine , infected dentine and affected dentine were placed in a small size capillary tube and were directly placed in the sample holder of instrument.

RESULTS AND DISCUSSIONS

Fig.1shows the infrared spectra of (a) hydroxyapatite, (b) sound dentine , (c) infected dentine and (d) affcted dentine in the wavenumber range of 500- 4000 cm^{-1}. In the spectrum of hydroxyapatite (and other spectra as well) the doublet band at 500 - 650 cm^{-1} is assigned to the υ_4 vibration of PO_4^{3-} [1,9]. The strong band at 900 - 1200 cm^{-1} is assigned to the υ_3 vibration of PO_4^{3-} . In the spectra labled b-d, two weak bands at 850 - 890 cm^{-1} and 1415 cm^{-1} are assigned to the υ_2 and υ_3 vibrations of CO_3^{2-} and three medium bonds at 1450, 1550 and 1660 cm^{-1} are assigned to amid vibrations of collagen [9].

It can be seen from Fig.1 that the spectrum of sound dentine (spectrum b) , except the collagen bands , is similar to the spectrum of hydroxyapatite (spectrum a) , but

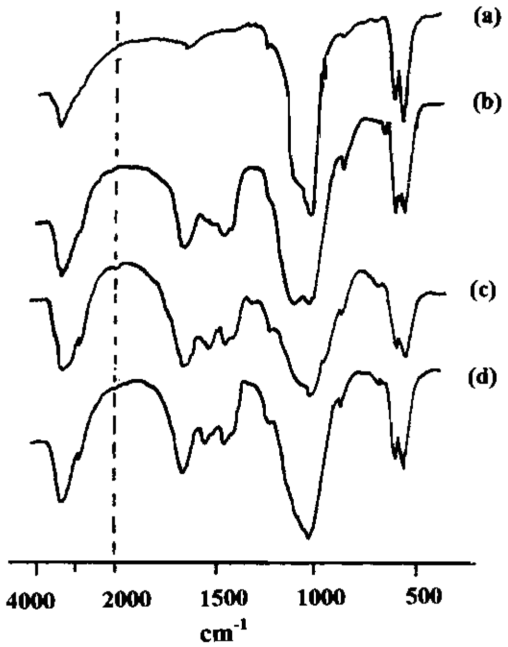

Fig.1: Infrared spectra of hydroxyapatite (a), sound dentine (b), infected dentine (c) and affected dentine (d) in the wavenumber range of 500- 4000 cm^{-1}

as we look at the spectrum of infected dentine (spectrum c) there is a large difference between the υ_3 vibration at this spectrum and that of dentine. The intensity of this band has been decreased whereas its width has increased. This may have arised from the conversion of hydroxyapatite to other phosphates such as ACP (Amorphous Calcium Phosphate) , TCP and DCPD (Dicalcium Phosphate Dihydrate) [10]. It shows that the infected dentine still has some mineralized phase. In a TEM study of infected dentine , Daculsi etal [6] concluded that in carious dentine the hydroxyapatite is converted to Mg - substituted - TCP. This is in good agreement with our IR study of infected dntine. In other hand the spectrum of affected dentine is more or less similar to that of sound dentine.

As far as the composition of collagen in infected and affected dentine is concerned, it can be seen from Fig.1 that the amid bands of collagen are closely similar to sound dentine. In these spectra, however, a new band has appeared at about 1500 cm^{-1} which might be due to a new structural modification of collagen in infected and affected dentine. These results are in agreement with Kuboki etal [5] which reported no significant change in the aminoacid composction of collagen in infected and affected dentine

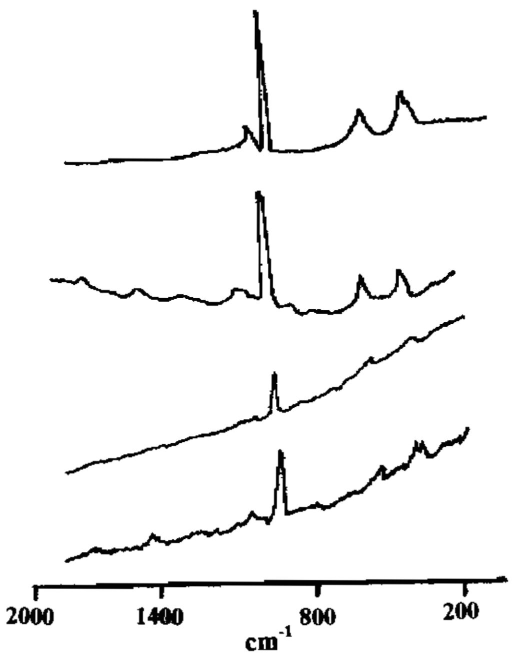

Fig.2: Raman spectra of hydroxyapatite (a), sound dentine (b), infected dentine (c) and affected dentine (d) in the wavenumber shift range 200-2000 cm^{-1}.

Fig.2 shows Raman spectra of sound dentine (b) , infected dentine (c) and affected dentine (d) in comparison with hydroxyapatite (a) in the wavenumber shift range 200-2000 cm^{-1}. It can be seen from Fig.2 that all spectra more or less show a similar pattern. The strong band at about 960 cm^{-1} is assigned to the symmtric stretching mode(υ_1)of the phosphate group [11]. The position of this band is slightly defferent in different phosphates. Values of 952 cm^{-1} for ACP , 951 cm^{-1} OCP (Octa Calcium Phosphate), 960 cm^{-1} for HAP , and 985 for DCPD have been reported [12]. In our Raman spectra shown at Fig.2 however, it is dificult to observe any small difference in the position of υ_1 vibration. We may only say that still infected dentine has a mineralized phase and affected dentine is closely similar to sound dentine.

The collagen bands in the Raman spectra are so weak in order to make any judgement.

CONCLUSIONS

On the basis of our IR and Raman deta it is concluded that infected dentine still has a mineralized phase and affected dentine has a similar structure to the sound dentine. In infected dentine the hydroxyapatite may have changed to other phosphates, most probable the TCP phase. The molecular structure of collagen fibers has not been changed in infected and affected dentine.

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