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Characterization of Human Teeth Using Vibrational Spectroscopies †

Ines Delfino [1,*], Marianna Portaccio [2], Giovanni Maria Gaeta [3] and Maria Lepore [2]

[1] Dipartimento di Scienze Ecologiche e Biologiche, Università della Tuscia, I-01100 Viterbo, Italy
[2] Dipartimento di Medicina Sperimentale, Università della Campania "L. Vanvitelli", Via S. Maria di Costantinopoli 16, I-80138 Napoli, Italy; marianna.portaccio@unicampania.it (M.P.); maria.lepore@unicampania.it (M.L.)
[3] Dipartimento di Scienze Odontostomatologiche e Maxillo Facciali, Università di Roma "La Sapienza", Via Caserta 6, I-00161 Roma, Italy; gmg.gaeta@gmail.com
* Correspondence: delfino@unitus.it
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Abstract: Dentin and enamel are the two main constituents of human teeth, and the detailed characterization of their biochemical properties is of fundamental relevance in many fields of dentistry research. Vibrational spectroscopies such as Fourier-Transform Infrared (FT-IR) spectroscopy and Raman spectroscopy can be adopted to obtain precise information before and after chemical or physical teeth treatments. In the present work, the two above-mentioned spectroscopic techniques were used to investigate dentin and enamel powders and few mm thick disks cut from human molar teeth. The FT-IR and Raman spectra clearly show the contributions of different sample components. The spectra obtained from the dentin and enamel powders evidence differences due to their chemical composition. The spectra from the human tooth disks present different characteristics depending on the region of the samples from which they were collected, thus enabling a spatial characterization of the samples themselves on different scales. These results confirm that vibrational spectroscopies allow a detailed characterization of hard dental tissues at the microscopic level.

Keywords: Fourier-Transform Infrared (FT-IR) spectroscopy; Raman spectroscopy; dentin and enamel powders; human molar tooth disk

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1. Introduction

Fourier-Transform Infrared (FT-IR) and Raman spectroscopies are two of the most common and up-to-date vibrational techniques, and are widely used in many applied research applications. In fact, these techniques are non-invasive, and often do not require complicated sample preparation procedures [1,2]. These techniques have been widely applied in dentistry to investigate the chemical compositions of different dental tissues and biofluids, and their changes induced by chemical or physical teeth treatments or pathological agents [3–6]. A particular relevant application of FT-IR and Raman spectroscopies in dentistry is related to the characterization of the chemical composition of dentin and enamel, the two main components of human teeth. In the present work, the two above-mentioned spectroscopic techniques were used to study dentin and enamel powders and few mm thick disks cut from human molar teeth. This investigation allowed us to optimize the measurement procedure for spectra acquisition, and to acquire essential information for investigating the chemical changes induced in hard dental tissues by endogenous or exogenous causes.

2. Materials and Methods

A selection of extracted teeth, removed for orthodontic and periodontal purposes, were disinfected in Sodium Hypochlorite 5% solution (Ogna Lab, Florence, Italy) for 24 h. Remnants of dental pulp tissue were removed. Slices a few mm thick were cut from

human molar teeth. The teeth were sectioned vertically with a diamond saw (Buehler, Lake Bluff, IL, USA). After preparation, the samples were stored in a dry state. Before laser processing, they were rehydrated with distilled water for 24 h to restore the normal fully hydrated state.

Tooth powder was obtained with a blue-ring angle handpiece (40,000 r/m) (Kavo Biberach, Biberach an der Riß, Germany), using a red-ring flame diamond bur, the Fine-Grit Diamond Milling Cutter FG314 (30 µm) (Komet, Verona, Italy). After preparation, the powder samples of enamel and dentin were stored separately, in a dry state, for the spectroscopic measurements.

FT-IR spectra of the powder samples were obtained by using a small amount of the samples and the Universal ATR (Attenuated Total Reflectance) accessory of a Perkin Elmer Spectrum One spectrometer (Perkin, Shelton, CT, USA), equipped with an MIR TGS detector. The spectra were acquired using 64 scans in the range of 4000 to 650 cm^{-1}, with a 4 cm^{-1} spectral resolution.

To analyze the tooth disks, the microscope stage of the previously mentioned spectrometer, equipped with a Mercury Cadmium Telluride (MCT) detector, was used to record micro-ATR spectra using a germanium hemispherical internal reflection element (IRE) with a 0.6 mm radius. Again, in this case, the spectra were acquired using 64 scans in the range of 4000 to 650 cm^{-1}, with a 4 cm^{-1} spectral resolution.

Raman measurements were carried out using a Horiba Xplora Raman micro-spectroscopy system, equipped with a Peltier-cooled CCD detector and a 785 nm laser with a maximum power of 100 mW. A 1200 lines/mm grating and a 50× objective were used. Spectra were acquired in the 600–3300 cm^{-1} range.

3. Results and Discussion

3.1. FT-IR Measurements of Dentin and Enamel Powder Samples

In Figure 1, the average spectra collected from the dentin powder samples are reported in the two panels (a) and (b), related to the high-wavenumber spectral range (3700–2000 cm^{-1}) and the fingerprint region (1800–800 cm^{-1}), respectively. Different contributions from the organic and inorganic dentin components are evident. The positions and the attributions of the most relevant peaks are reported in Table 1.

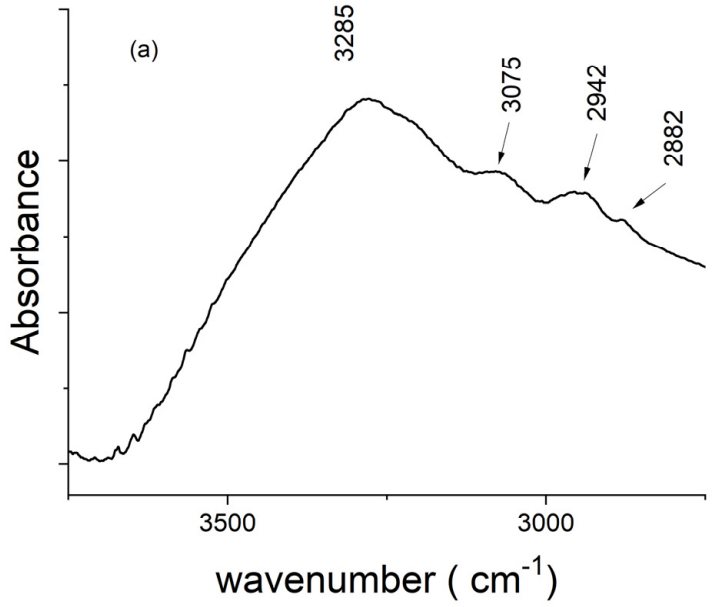

Figure 1. *Cont.*

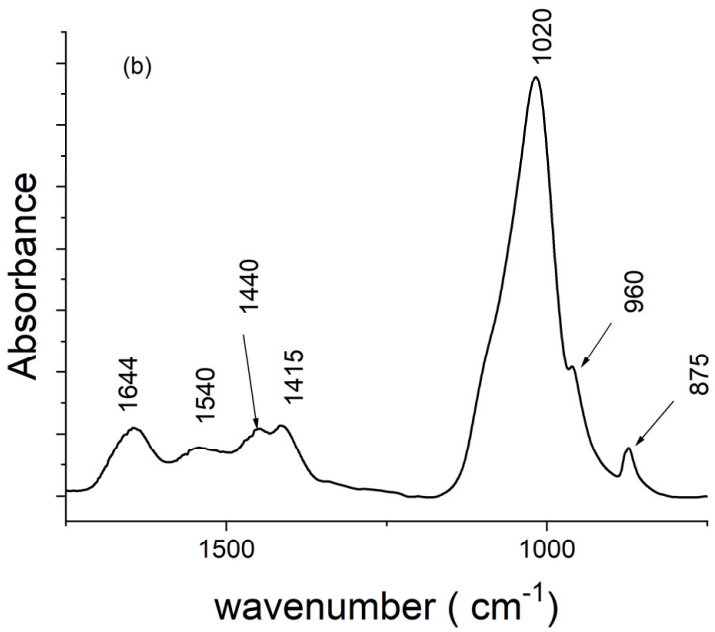

Figure 1. Average FT-IR spectra of dentin powder samples, separately reported for high-wavenumber region (**panel a**) and fingerprint region (**panel b**).

Table 1. Main peaks in FT-IR spectra of dentin and enamel powder samples, with their assignments in agreement with references. [7–9].

Dentin Powder Peak (cm^{-1})	Enamel Powder Peak (cm^{-1})	Assignments	Components
3285		Hydroxyl O-H stretching N-H stretching-Amide A	Carbonated hydroxyapatite Protein
3075			
2942–2882		C-H bond stretching	Lipid
1644		Amide I	Protein (collagen)
1540		Amide II	Protein (collagen)
1440	1457	C-O ν_3 stretching	CO_3 Carbonated hydroxyapatite
1415	1412	Amide III	Protein (collagen)
1020	1015	P-O ν_1 symmetric stretching	PO_4 Carbonated hydroxyapatite
960	956	C-O ν_2 bending	CO_3 Carbonated hydroxyapatite
875	876		

In Figure 2, the average spectrum collected from the enamel powder samples is reported. The few relevant peaks present in the spectrum are listed in Table 1, together with their assignments.

The FT-IR average spectra of the two main components of dental tissue allow them to be characterized, and can be useful for interpreting the spectra obtained from the molar disk samples, as reported in the following paragraph.

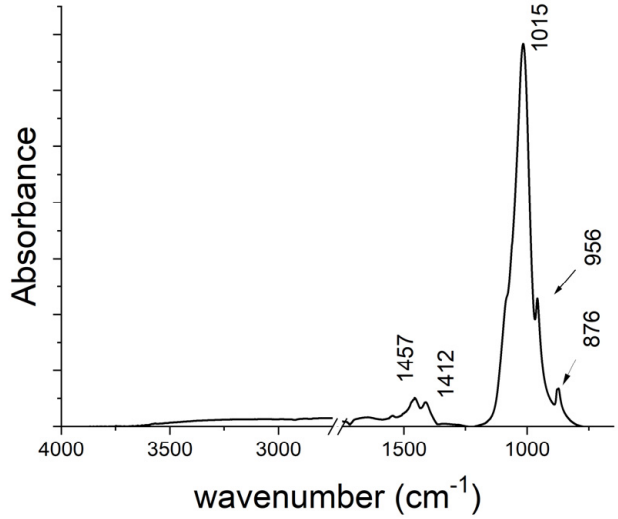

Figure 2. Average FT-IR spectrum of enamel powder samples.

3.2. FT-IR Measurements of Human Molar Disks

In Figure 3, the FT-IR spectra acquired from different regions of the human molar disks are reported. In this case, the use of the germanium IRE allows the characterization of small areas of the samples with a spatial resolution of tens of μm^2. In this way, the changes induced in the chemical composition of small areas of the teeth by treatments with physical and chemical external agents can be easily investigated [7–11].

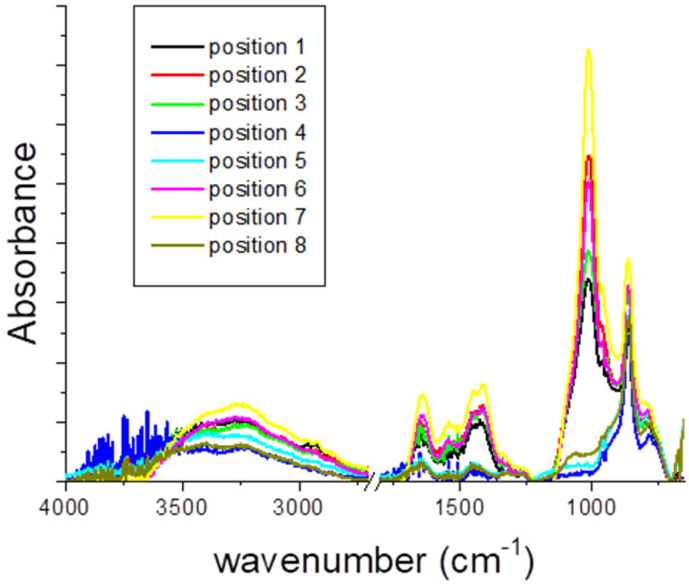

Figure 3. FT-IR spectra collected from different regions on the surface of a human molar disk.

3.3. Raman Measurements of Dentin and Enamel Powder Samples

A representative spectrum collected from the dentin powder samples, reported for the 600–1800 cm^{-1} spectral region, is shown in Figure 4. Some contributions from organic and inorganic dentin components are evident. In Figure 5, a representative spectrum collected from the enamel powder samples is reported, showing a peculiar relative intensity of the observed peaks. The main Raman peaks of enamel and dentin powder reported in the literature are listed in Table 2.

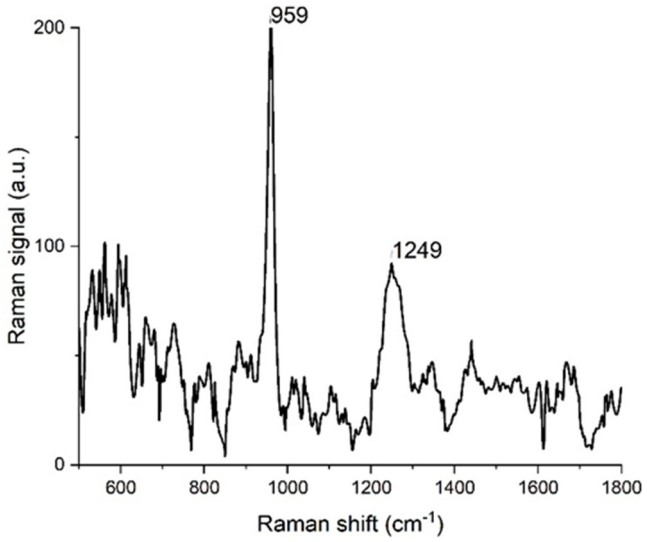

Figure 4. Representative Raman spectrum of dentin powder samples.

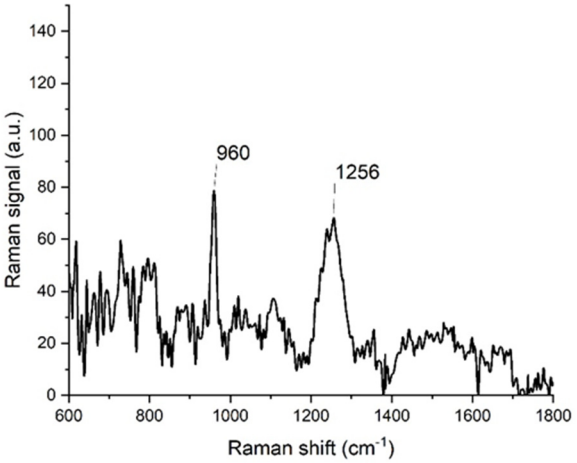

Figure 5. Representative Raman spectrum of enamel powder samples.

Table 2. Main peaks in Raman spectra of dentin and enamel powder samples, with their assignments [12–15].

Dentin Powder Peak (cm^{-1})	Enamel Powder Peak (cm^{-1})	Assignments	Components
1660–1665		Amide I	Protein (collagen)
1520		Amide II	Protein (collagen)
1450		C-O ν_3 stretching	CO_3 Carbonated hydroxyapatite
1240–1247	1250	Amide III	Protein (collagen)
1025; 1045	1025; 1045	P-O ν_1 symmetric stretching	PO_4 Carbonated hydroxyapatite
960	960	C-O ν_2 bending	CO_3 Carbonated hydroxyapatite

3.4. Raman Measurements of Human Molar Disks

Some Raman spectra obtained from different positions on the molar disk samples are shown in Figure 6. Contributions of the two main components of the teeth can be observed, with the relative intensities of the various peaks being highly dependent on the investigated positions. This confirms the ability of Raman spectroscopy to investigate

tooth samples. In addition, taking advantage of the higher spatial resolution offered by this technique, it is possible to investigate micrometric regions. The Raman spectra reported in Figures 4–6 allow the identification of different spectral contributions, even though they have been preliminarily obtained from the raw data by simply subtracting the fluorescence background signals that are generally present when biological samples are examined. It is well known that the quality of Raman spectra can be significantly improved by applying noise reduction algorithms (see Ref. [16] and the references therein).

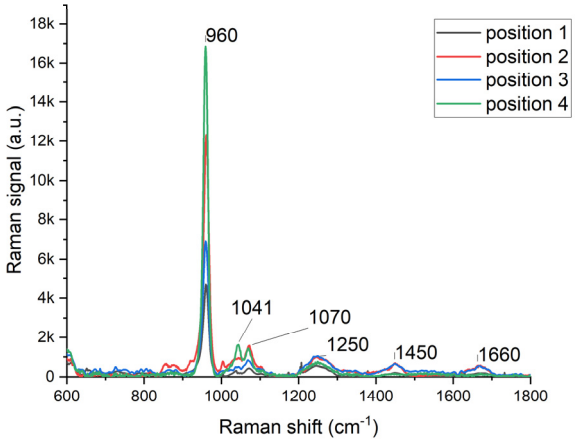

Figure 6. Raman spectra collected from different regions on the surface of a human molar disk.

4. Conclusions

The present investigation allowed us to optimize the measurement procedures for spectra acquisition, and confirmed that FT-IR and Raman spectroscopies represent useful tools for characterizing the chemical composition of hard dental tissues. The two above-mentioned techniques have been demonstrated to give complementary information that can be particularly valuable when teeth undergo different chemical and physical treatments. In these cases, the high sensitivity of FT-IR spectroscopy and the excellent spatial resolution of Raman spectroscopy can jointly contribute to obtaining a precise and detailed description of the changes induced and the processes occurring during treatments.

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