engineering proceedings

MDPI

Proceeding Paper

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, 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, 6-00161 Roma, Italy; gmg.gaeta@gmail.com
* Correspondence: delfino@unitus.it
† Presented at The 11th International Electronic Conference on Sensors and Applications (ECSA-11), 26–28 November 2024; Available online: https://sciforum.net/event/ecsa-11.

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) 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 have been used for investigating dentin and enamel powders and few-mm thick disks cut from human molar teeth. FT-IR and Raman spectra clearly show the contributions of different sample components. The spectra obtained from dentin and enamel powders evidence the differences due to their chemical composition. The spectra from 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

Citation: Delfino, I.; Portaccio, M.; Gaeta, G.M.; Lepore, M. Characterization of Human Teeth Using Vibrational Spectroscopies. *Eng. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: 26 November 2024

1. Introduction

Fourier Transform Infrared (FT-IR) and Raman spectroscopies are two of the most common and up-to-date vibrational techniques largely used in many applied research applications. In fact, these technique are non-invasive and often do not require complicate sample preparation procedures [1,2]. These techniques have been largely applied in dentistry to investigate the chemical composition 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 spectroscopy 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 have been 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 occurring in hard dental tissues by endogenous or exogenous causes.

2. Materials and Methods

A selection from extracted teeth removed for orthodontic and periodontal purposes were disinfected in Sodium Hypochlorite 5% solution (Ogna Lab, Florence Italy) for 24 Hours. Remnants of dental pulp tissue were removed. Few-mm thick slices 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.

The teeth powder was obtained with angle handpiece blue ring 40,000 r/m (Kavo Biberach, Germany) using a flame red ring diamond bur Fine-Grit Diamond Milling Cutters 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 powder samples were obtained by using small amount of the samples and the Universal ATR (Attenuated Total Reflectance) accessory of a Perkin Elmer Spectrum One spectrometer equipped with a MIR TGS detector. The spectra were acquired using 64 scans in the range from 4000 to 650 cm^{-1} with a 4 cm^{-1} spectral resolution.

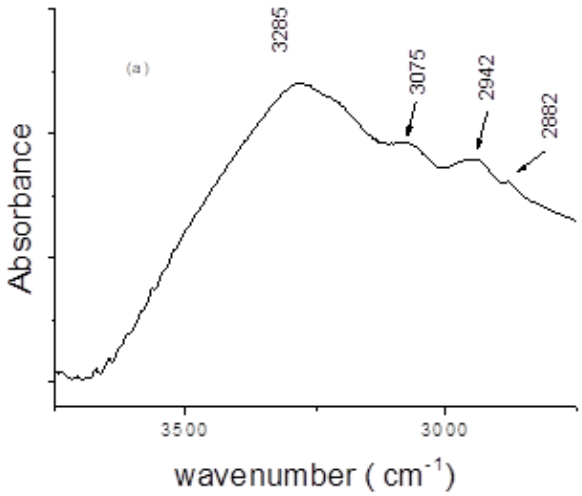
To analyze tooth disk the microscope stage of the previously mentioned spectrometer equipped with a Mercury Cadmium Telluride detector (MCT) was used to record micro-ATR spectra by using a 0.6 mm radius germanium hemispherical internal reflection element (IRE). Also in this case, the spectra were acquired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

Raman measurements were carried on by a Horiba Xplora Raman micro-spectroscopy system equipped with a Peltier cooled CCD detector, 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 on Dentin and Enamel Powder Samples

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



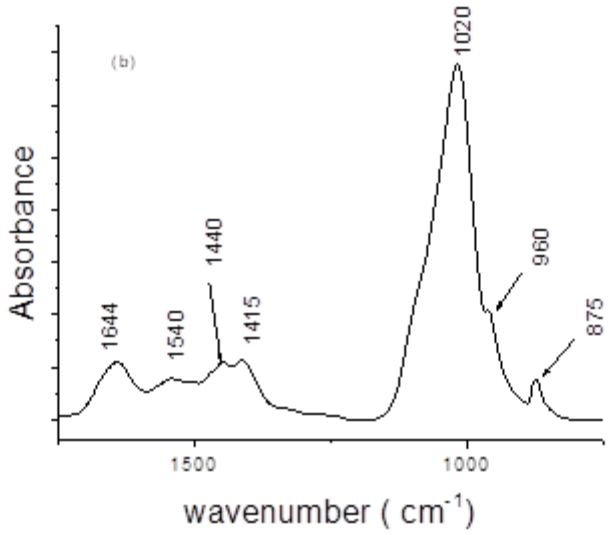


Figure 1. Average FT-IR spectrum of dentin powder samples, separately reported for high wavenumber region (panel **a**) and fingerprint region (panel **b**).

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

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

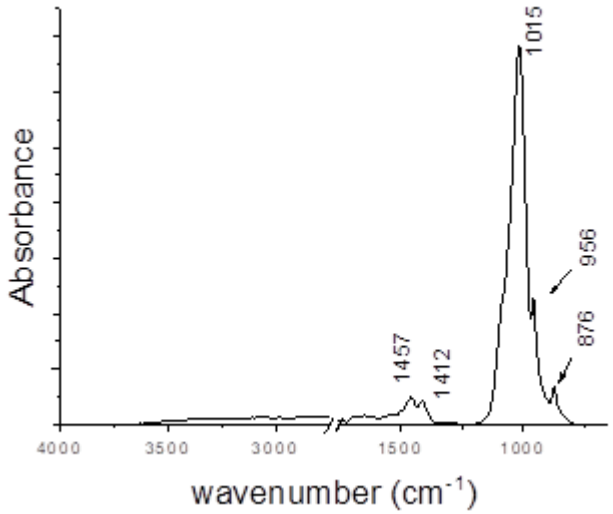


Figure 2. Average FT-IR spectrum of enamel powder samples.

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

Dentin Powder Peak (cm^{-1})	Enamel Powder Peak (cm^{-1})	Assignments	Components
3285		Hydroxyl stretch O-H stretching N-H stretching- Amide A	Carbonated hydroxyapatite Protein
3075			
2942–2882		C-H bonds 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		

3.2. FT-IR Measurements on Human Molar Disks

In Figure 3 the FT-IR spectra acquired from different regions in a 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 teeth by treatments with physical and chemical external agents can be easily investigated [7–11].

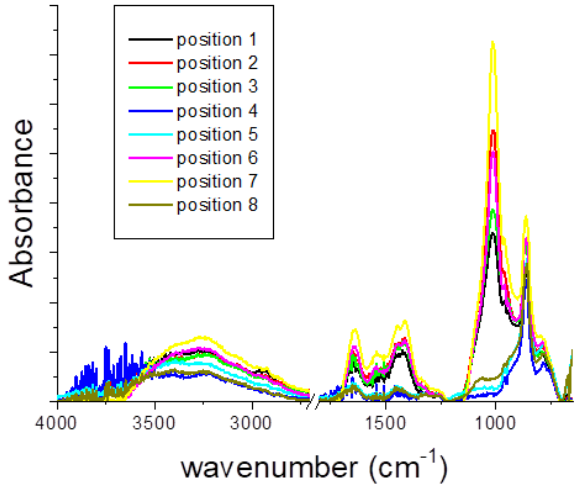


Figure 3. FT-IR spectra collected from different regions on the surface of a human molar disk.

3.3. Raman Measurements on Dentin and Enamel Powder Samples

A representative spectrum collected from dentin powder samples is 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 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 literature are listed in Table 2.

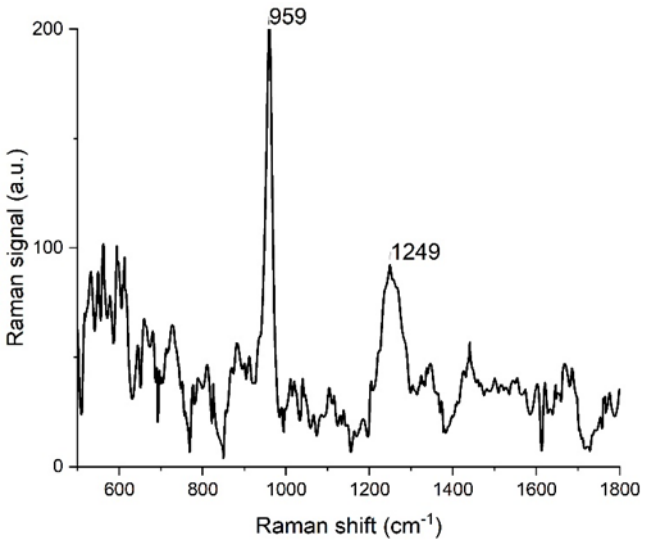


Figure 4. Representative Raman spectra of dentin powder samples.

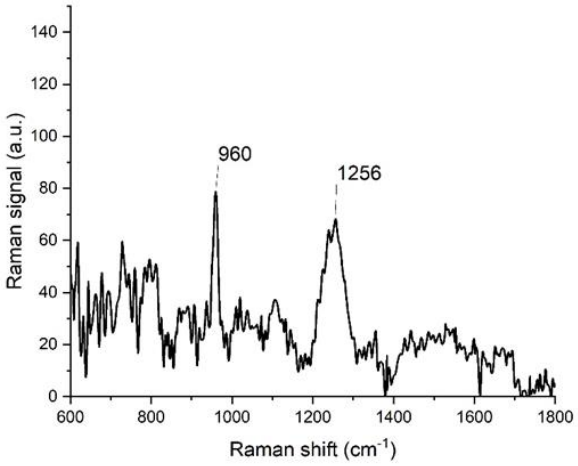


Figure 5. Representative Raman spectra of enamel powder samples.

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 on Human Molar Disk

Some Raman spectra obtained from different positions of molar disk samples are shown in Figure 6. The contributes due to the two main components of the teeth are observed, the relative intensity of the various peaks being highly dependent on the investigated positions. This confirmed the ability of Raman spectroscopy to investigate teeth samples. In addition, taking advantage of higher spatial resolution offered by this

technique, it is possible to investigate micrometric regions. Raman spectra reported in Figures 4–6 allow the identification of different spectral contributions even though they have been preliminarily obtained from 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 refences therein).

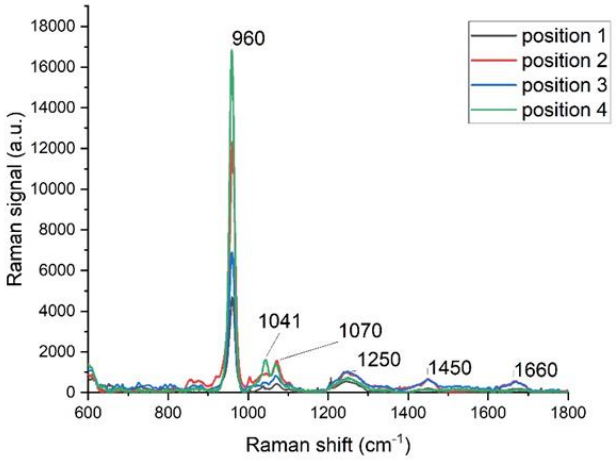


Figure 6.

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 are 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 obtain a precise and detailed description of the changes induced and the processes occurring during the treatments.

Author Contributions: Conceptualization, M.L., M.P., I.D. and G.M.G.; data curation, M.L., M.P. and I.D.; sample preparation, G.M.G.; writing, M.L., M.P., I.D. and G.M.G.; writing—review and editing, M.L., M.P. and I.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement:

Informed Consent Statement: Not applicable.

Data Availability Statement:

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Beć, K.B.; Grabska, J.; Huck, C.W. Biomolecular and bioanalytical applications of infrared spectroscopy: A review. *Anal. Chim. Acta* **2020**, *1133*, 150–177.
2. Butler, H.J.; Ashton, L.; Bird, B.; Cinque, G.; Curtis, K.; Dorney, J.; Esmonde-White, K.; Fullwood, N.J.; Gardner, B.; Martin-Hirsch, P.L.; et al. Using Raman spectroscopy to characterize biological materials. *Nat. Protoc.* **2016**, *11*, 664–687.
3. Alebrahim, M.A.; Krafft, C.; Popp, J. Raman imaging to study structural and chemical features of the dentin enamel junction *IOP Conf. Ser. Mater. Sci. Eng.* **2015**, *92*, 012014. https://doi:10.1088/1757-899X/92/1/012014.

4. d'Apuzzo, F.; Nucci, L.; Delfino, I.; Portaccio, M.; Minervini, G.; Isola, G.; Serino, I.; Camerlingo, C.; Lepore, M. Application of Vibrational Spectroscopies in the Qualitative Analysis of Gingival Crevicular Fluid and Periodontal Ligament during Orthodontic Tooth Movement. *J. Clin. Med.* **2021**, *10*, 1405. https://doi.org/10.3390/jcm10071405.
5. Diez, C.; Rojo, M.A.; Martín-Gil, J.; Martín-Ramos, P.; Garrosa, M.; Córdoba-Diaz, D. Infrared Spectroscopic Analysis of the Inorganic Components from Teeth Exposed to Psychotherapeutic Drugs. *Minerals* **2022**, *12*, 28.
6. Otel, I. Overall Review on Recent Applications of Raman Spectroscopy Technique in Dentistry. *Quantum Beam Sci.* **2023**, *7*, 5. https://doi.org/10.3390/qubs7010005.
7. Alves, S.; Oliveira, V.; Vilar, R.; Femtosecond laser ablation of dentin. *J. Phys. D Appl. Phys.* **2012**, *45*, 24540.
8. Le, Q.T.; Bertrand, C.; Vilar, R. Structural modifications induced in dentin by femtosecond laser. *J. Biomed. Opt.* **2016**, *21*, 125007.
9. Petrov, T.; Pecheva, E.; Walmsley, A.D.; Dimov, S. Femtosecond laser ablation of dentin and enamel for fast and more precise dental cavity preparation. *Mater. Sci. Eng. C* **2018**, *90*, 433–438.
10. Beddoe, M.; Gölz, T.; Barkey, M.; Bau, E.; Godejohann, M.; Maier, S.A.; Keilmann, F.; Moldovan, M.; Prodan, D.; Ilie, N.; et al. Probing the micro- and nanoscopic properties of dental materials using infrared spectroscopy: A proof-of-principle study. *Acta Biomater.* **2023**, *168*, 309–322. https://doi.org/10.1016/j.actbio.2023.07.017.
11. Elfarraj, H.; Lizzi, F.; Bitter, K.; Zaslansky, P. Effects of endodontic root canal irrigants on tooth dentin revealed by infrared spectroscopy: A systematic literature review. *Dent. Mater.* **2024**, *40*, 1138–1163. https://doi.org/10.1016/j.dental.2024.05.014.
12. Hikov, T.; Pecheva, E.; Montgomery, P.; Antoni, F.; Leong-Hoi, A.; Petrov, T. Precise femtosecond laser ablation of dental hard tissue: Preliminary investigation on adequate laser parameters. *J. Phys. Conf. Ser.* **2017**, *794*, 012036.
13. Rapp, L.; Madden, S.; Brand, J.; Walsh, L.J.; Spallek, H.; Zuaiter, O.; Habeb, A.; Hirst, T.R.; Rode, A.V. Femtosecond laser dentistry for precise and efficient cavity preparation in teeth. *Biomed. Opt. Express* **2022**, *13*, 4559–4571.
14. Le, Q.T.; Bertrand, C.; Vilar, R. Femtosecond laser ablation of enamel. *J. Biomed. Opt.* **2016**, *21*, 065005.
15. Rapp, L.; Madden, S.; Brand, J.; Maximova, K.; Walsh, L.J.; Spallek, H.; Zuaiter, O.; Habeb, A.; Hirst, T.R.; Rode, A.V. In-vestigation of laser wavelength effect on the ablation of enamel and dentin using femtosecond laser pulses. *Sci. Rep.* **2023**, *13*, 20156. https://doi.org/10.1038/s41598-023-47551-5.
16. Camerlingo, C.; Zenone, F.; Gaeta, G.M.; Riccio, R.; Lepore, M. Application of wavelet data treatment on micro-Raman spectra of biological samples. *Meas. Sci. Technol.* **2006**, *17*, 298–303.