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Mid-infrared reflectance microspectroscopy of human molars: Chemical comparison of the dentin–enamel junction with the adjacent tissues

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ABSTRACT

Mid-infrared reflectance microspectroscopy was applied to the study of calcified tissues in human molars. Particular attention was focused on the dentin–enamel junction (DEJ). Major chemical components (apatite, organic matrix and water) and minor chemical components (CO_3^{2-} and HPO_4^{2-} ions in apatite) were investigated. It was found that the average contents of organic matrix and CO_3^{2-} ions increased in the order: enamel < DEJ < dentin. The smallest quantities of water and phosphates were observed in the DEJ, which finding can be explained by the loosest tissue packing in this location. The HPO_4^{2-} ions were not detected in the DEJ, while in dentin their content was higher than in enamel. Differences between the reflection and transmission modes in the studies of calcified tissues were discussed.

1. Introduction

Spatial variations in constituents of biological tissues are crucial determinants of their functions in health and disease. Therefore, the biological tissues should be examined from very small compartments in a position-resolved way and with the least possible preparation to prevent structural modifications. Such bioanalytical conditions are feasible with Fourier-transform infrared microspectroscopy (FT-IRM) [1,2] offering spatial resolution down to ca. 10 μm and typical spectral resolution of 1–5 cm^{-1}. The FT-IRM technique can be used in transmission, reflectance and attenuated total reflection (ATR) modes. It is always coupled to a light microscope, which allows the investigator to select discrete spots or larger fragments of a sample for the point-by-point analysis or mapping, respectively. FT-IRM has already been applied to study various mineralized tissues, such as bone, calcified cartilage, dental enamel, dentin and cementum [3–27]. So far, human dental tissues have rarely been analyzed [20–22,25].

Enamel and dentin have dissimilar mechanical, chemical and morphological characteristics as well as unlike embryogenic origins [28,29]. The dentin–enamel junction (DEJ) [30] unites those unique, composite biomaterials. The DEJ is regarded by most authors as a transitional zone or an interphase between two dental structures or as a border involving the inner aprismatic enamel and the mantle dentin [31–38]. Its width is greatly dependent on the analytical method and the reported values are within a 2–100 μm range [32]. The DEJ is remarkably competent in transferring large repeated masticatory and impact loads from enamel to dentin over decades of life [39]. Furthermore, flaws and cracks coming from enamel are often deflected or arrested in the DEJ, so they do not penetrate deeply into dentin [31,32,38,40,41]. Hence, the tooth integrity is retained.

The knowledge of the chemical structure of the DEJ is still insufficient, although many significant papers were published on the chemical composition of dentin and enamel, and its variation across those tissues [21–23,26,27,42–54]. Thus, considering the previous studies, one would expect crystallinity of apatite mineral to reach maximum at the DEJ, while the mineral and HPO_4^{2-} contents to be at a minimum. Coming from the circumpulpal dentin side, the relative organic matrix/mineral ratio was found to increase towards the DEJ, reaching a maximum in the mantle dentin and then to decrease rapidly across the junction. For water it is only known that its concentration in dentin decreases towards the DEJ. The structural OH^- content should experience a sharp rise from dentin to enamel across the DEJ. The literature information concerning carbonates on the dentin side is inconclusive, while in enamel the CO_3^{2-} concentration decreases from the DEJ. Carbonate ions can replace OH^- or PO_4^{3-} ions in the calcium hydroxyapatite structure, giving substitutions of type A and B, respectively. In addition, labile carbonate ions can exist on the apatite crystal surface. It is rather accepted, that the dentin mineral is carbonatoapatite of type B, while substitutions A, B and mixed AB are more balanced in the enamel mineral. The chemical/molecular structure

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of organic matrix in the DEJ is probably different from that in bulk dentin and dependent on the intratooth location [54]. Overall, the DEJ is less mineralized than either enamel or bulk dentin, contains more organic matrix and is probably associated with the first formed mantle dentin [41].

In the case of methods having good spacial resolution, an abrupt chemical alteration within the DEJ was observed. In particular, Raman microspectroscopic studies of human teeth showed a sharp dentin-to-enamel intensity change of the bands representing organic matrix (decrease) and apatite mineral (increase) [41,43,54,55]. Similarly, it was found that the mineral:matrix FT-IR parameter increased in a steep manner across the DEJ when passed from dentin to enamel (bovine sample) [27]. By contrast, according to SEM energy dispersive spectroscopy (SEM-EDS), within the DEJ zone of a human tooth there is a prominent dip in the monotonic dentin-to-enamel increase of the mineral content [38].

The above short assessment of the DEJ chemical features indicates that there is still necessity to continue research in this scientific field. To the best of our knowledge, in the dental studies the reflectance FT-IR microspectroscopy has been used only once by Tesch et al. [22] and exclusively to investigate human dentin. Our work is rather aimed at the chemical comparison of the DEJ to both adjacent tissue regions in human molars. The topic is important for structural biology, dentistry and material science.

2. Materials and methods

The study was performed on three non-carious molars (M3) extracted with informed consent from adult patients undergoing routine orthodontic extraction at the Department of Dental Surgery of the Medical University of Warsaw. Each tooth had its pulp removed, and then it was wiped clean and dried in air at room temperature. Afterwards, the roots were cut out and the crown was sliced with an EDENTA disc cutter covered with a diamond grit (mesh 105–125 μm) to prepare a 500 μm thick, longitudinal mid-crown section perpendicular to the buccal and lingual surfaces. Finally, the section was cautiously polished with Sof-Lex abrasive discs (3M Company) to remove any surface roughness. The samples prepared of those three molars extracted from separate donors have been designated S1, S2 and S3.

The mid-IR FT surface spectra were recorded with a Perkin-Elmer Autoimage IR microscope operating in the reflection mode (specular reflectance) and equipped with a conventional MCT detector with the low frequency cut-off at 450 cm^{-1}. The microscope was coupled to a Spectrum 1000 Perkin-Elmer Spectrometer. The spectra were acquired from square pixels 50 × 50 μm^2, with 1 cm^{-1} spectral resolution, using 1000 scans. They were processed with the GRAMS/AI 8.0 software (Thermo Scientific), which was also used for integration, differentiation and deconvolution of selected spectral regions, and curve fitting of overlapping bands. Selected fittings were done using the ACD software. The spectra were subjected to the Kramers–Kronig transformation. The air background had been recorded using a gold mirror in the same conditions as for the studied sample and then it was subtracted from each spectrum. The spectra were measured from 10 different sites along the optical DEJ and from 18 different sites across the DEJ by moving outwards throughout dentin (7 spectra) and enamel (10 spectra) as shown in Fig. 1. The distances given in Figs. 5–8 correspond to centers of the square pixels, from which the spectra were recorded. The scanning direction for S1–S3 was deliberately chosen for two reasons. First, we have decided to select the crown regions with sound outer enamel to be sure that underlying tissues did not undergo any mechanical and/or chemical lesions. Second, we wanted to follow such a route, for which the widths of dentin and enamel were approximately the same, here ca. 1400 and 1600 μm, respectively. In order to get comparative spectra to the average DEJ spectrum, we have recorded them from large pixels of dentin and enamel (200 × 200 μm^2, resolution 3 cm^{-1}) at the distances of 800 μm from the DEJ. The results presented in Tables 1–4 were obtained using the spectra given in Fig. 2. The representative optical microscopic image of the studied crown section is given in Electronic Supplementary Material.

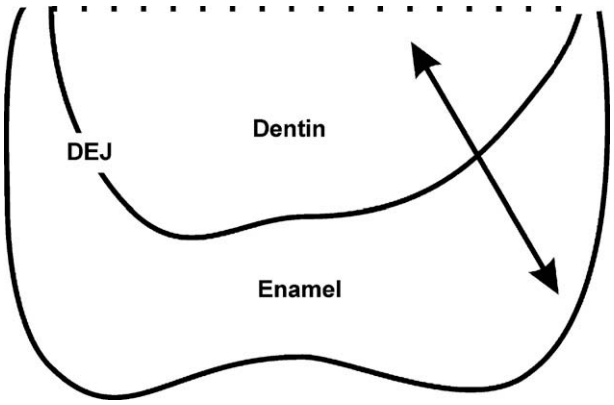

Fig. 1. A schematic drawing of the analyzed slice of a human molar (sample S1; longitudinal mid-crown section perpendicular to the buccal and lingual surfaces). The FT-IRM spectra of the DEJ were measured from 10 different sites along the DEJ and then an averaged DEJ spectrum was computed. The double-arrow line represents the transverse route of the FT-IRM sampling by moving away from the DEJ throughout dentin and enamel.

Table 1
Main IR bands from calcified tissues. For comparison, in the square brackets there are results for pure collagen from this work (collagen from bovine tendon, powder, reflectance spectrum).

Wavenumber (cm^{-1})	Assignment
Mineral (apatite)	
3572	Stretching OH^-
1545	ν_3 CO_3^{2-} type A
1465	ν_3 CO_3^{2-} type B
1450	ν_3 CO_3^{2-} type A
1419	ν_3 CO_3^{2-} type B
1200–900	$\nu_1 + \nu_3$ PO_4^{3-} and HPO_4^{2-}
890–850	ν_2 CO_3^{2-} both types
870	HPO_4^{2-}
650–500	ν_4 PO_4^{3-}
630	Librational OH^-
Organic matrix (proteins)	
ca. 3300 [3284[a]]	Amide A
ca. 3100 [3079]	Amide B
1700–1600 [1655]	Amide I
1600–1500 [1543]	Amide II
1465 [1454]	CH_2 scissoring CH_3 asymmetric deformation
1400 [1401]	C—O stretching in carboxylates (amino acid side chains)
1200–1350 [1238]	Amide III
[1162 and 1088]	Stretching: C—O and C—C
[1030]	α-amino carboxylates
Water (hydrogen bonded)	
3800–2800 with max. at ca. 3300–3500	$\nu_1 + 2\nu_2 + \nu_3$ very strong and broad
1640	ν_2 bending

[a] On top of a massive water band.