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Root Dentin Analysis from using Fourier-Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR)

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Abstract: ***Dentin is the structure that greatly influences the teeth's resistance to masticatory forces in both norm and pathology. The aim of our study was to determine the changes that take place in samples of root dentin in vital and devitalized teeth using FTIR-ATR. The samples used were taken from young root dentin, old root dentin and root dentin from endodontically treated teeth (ETT). The investigations were conducted using an infrared spectrometer FTIR Tensor 37 Bruker in the middle infrared spectre (400 – 4000 cm^{-1}) with an ATR sensor attachment. The spectroscopic images obtained showed typical spectres for dentin, with absorbance peaks between 800 – 3500/cm^{-1}. The results showed that the hydroxyapatite content in root dentin is decreased and the collagen structure is changed. Aging and endodontic treatment could lead to changes in root dentin that affect both the hydroxyapatite and the collagen structure. Therefore, endodontic treatment could potentiate mechanisms, similar to those observed during the physiologycal aging of dentin. In contrast to aging though, the process is sped up and affects mainly the root dentin.***

Keywords: FTIR, ATR, dentin, spectroscopy, dentin

1. Introduction

Dentin is the structure that greatly influences the teeth's resistance to masticatory forces in both norm and pathology. Knowledge of its mechanical properties can contribute to the development of appropriate strategies for the restoration of their normal functions.Endodontically treated teeth (ETT) are more susceptible to vertical fractures that have a poor prognosis and make the success of further restorations questionable. The possible reasons include a decrease in the water content, especially a reduction in the unbound water [1,2]. Ultrastructural changes in the dentin are also discussed: especially disruptions in the covalent bonds in collagen that normally ensure dentin's stability and tensile strength [3].

2. Literature Survey

In recent years there has been a surge in the introduction of new methods in dental research that, up until now, have been mainly used in the fields of chemistry, physics, mineralogy, pharmaceutics and engineering sciences. Nanoindentation was used for assessing the mechanical properties of hard dental tissues, treated under different conditions [4,5]. X-ray diffraction could help in analyzing the properties of newly developed materials and the tracing of phase changes [6,7]. The different subtypes of infrared spectroscopy can also be used successfully: Fourier-transform (FTIR), Fourier-transform with attenuated total reflectance (FTIR-ATR), Raman spectroscopy. They all use the energy from infrared electromagnetic waves in the range between 300 MHz – 3 GHz. The molecules of the analyzed samples absorb part of the electromagnetic radiation. The absorbtion intensity varies according to the frequency of the electromagnetic waves and those variations form the abosrbance spectre of the sample. This can help in detereming its chemical contents, proving the existance of a certain compound and its quantities. A great advantage of the method is that the samples can be in liquid, solid or gaseous states [8].

Problem definition

The aim of our study was to determine the changes that take place in samples of root dentin in vital and devitalized teeth using FTIR-ATR.

3. Methodology/ Approach

Material – sample selection and preparation

The samples used in our study were taken from crown dentin of intact extracted human teeth. The teeth were ultrasonically cleaned and stored in a 0.2% thymol solution at a temperature of 4 ^{o}C for a period of up to 3 months. The "young dentin" sample was taken from impacted third molars in patients aged 18-30 years. The "old dentin" sample was taken from premolars, extracted due to general periodontitis in patients aged 40-65. The "endodontically treated" dentin sample was taken from teeth that had undergone endodontic treatment prior to extraction. The groups are represented in Table 1. Each sample was embedded in epoxy resin and polished to a surface roughness of up to 50 nm.

Table 1: Distribution of the studied groups.

	Young dentin	Old dentin	Dentin from an ETT
Root dentin	G4	G5	G6

4. Method

The investigations were conducted using an infrared spectrometer FTIR Tensor 37 Bruker in the middle infrared spectre (400 – 4000 cm^{-1}) with an ATR sensor attachment. For each sample, three investigation areas on the dentin surface were randomly selected. The samples were mounted on a standard FTIR holder with a 5 mm opening. During the study, the ATR-FTIR spectres of air were registered and automatically substracted with the program Opus. The ratios between the combined integrated areas of peaks v_1PO_4 and v_3PO_4 to the amide I peak were calculated for each spectre. This represented the hydroxyapatite/collagen contents in each of the investigated three areas. The average values were calculated and statistically analyzed.

5. Statistical Analysis

The statistical analysis was carried out using analysis of variance (ANOVA) in combination with Post-Hoc analysis. The independent variable was "dentin type", and the dependent one – "ratio between phosphate/amide groups". The software used was SPSS 16.0 (SPSS Inc, Chicago, IL).

6. Results & Discussion

The typical spectre of an untreated, intact dentin is shown on Fig. 1. The observed spectres are marked in the range between 800 – 3500/cm^{-1} according to the data in the available literature [9,10]. These peaks correspond to the vibrations of certain chemical bonds of the 3 major dentin components – hydroxyapatite, collagen and water. A representative spectre for each of the investigated groups is shown on Fig. 2 (G1 – G3). The calculated areas of peaks v_1PO_4, v_3PO_4 and amide I with their ratios (phosphate/amide groups) are shown on Table 2. They are graphically represented in a descending order on Fig. 3.

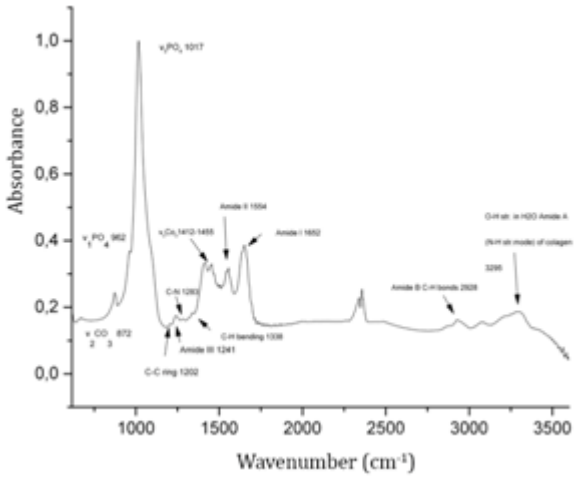

Figure 1: Typical infrared dentin spectre. The absorbance peaks between 800 – 3500/cm^{-1} are marked with arrows.

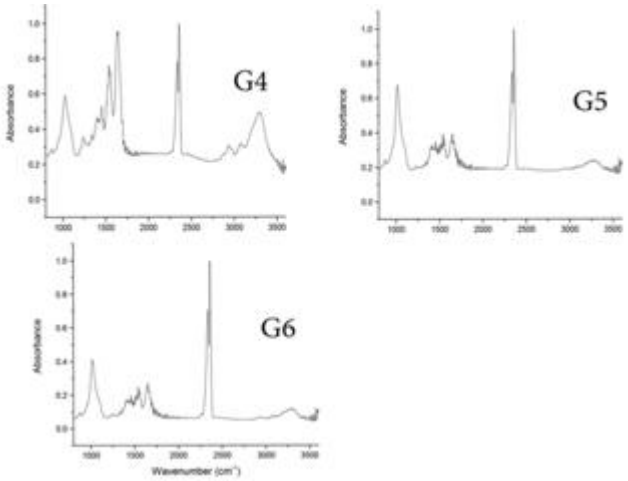

Figure 2: Spectograms of "young crown" dentin (G4), "old crown" dentin (G5), "devitalized crown" dentin (G6).

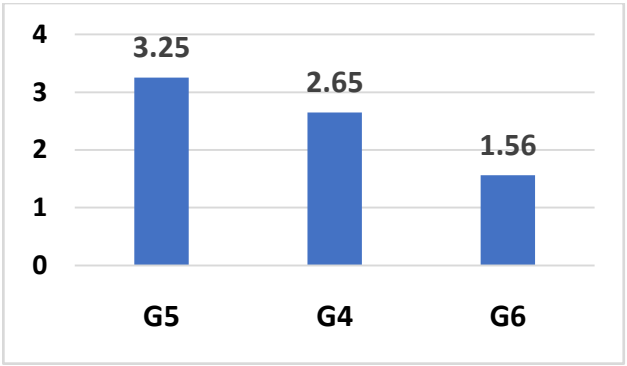

Fig. 3. Comparison of "phosphate/amide" groups ratios for all samples (G4 – G6).

The absorbance spectres of dentin in our study correspond to those in the available literature. Each spectre consists of those of dentin's main components: hydroxyapatite, collagen and water. Hydroxyapatite (pure and carbonated) is represented by phosphate, carbonate and hydroxylene bonds, which are graphically shown as peaks on the resultant spectograms (Fig. 2) [11, 13].

Collagen is represented by amide (-NHCO-), C-N, C-H, C-C and N-H bonds [14]. Water is found in two states – loosely bound (absorbed) and strongly bound (embedded in the structure of dentin). Hydroxylene bonds that correspond to the water molecules have the highest intensity between 3100-3398 cm^{-1}. They are observed in all of the studied groups, with the most intensity in the "young root dentin" group (G4). A possible explanation could be found in the changes that occur in the hydroxyapatite crystals with aging and endodontic treatment.

Table 2: Area values of peaks v_1PO_4, v_3PO_4 and amide I for each investigated area on the samples' surface.

G	Chosen area on the sample surface	Area values of peak v_1PO_4 (a)	Area values of peak v_3PO_4 (b)	Area values of peak Amide I (c)	Inorganic/ organic ratio (a+b)/c
G 4	1	5.005	40.622	12.258	3.722
	2	4.310	43.669	12.875	3.727
	3	1.034	13.995	30.151	0.498
G 5	1	4.781	42.514	14.501	3.261
	2	2.788	33.573	11.833	3.073
	3	4.043	36.810	11.945	3.420
G 6	1	4.674	39.719	19.654	2.259
	2	2.761	23.653	19.664	1.343
	3	2.827	24.684	25.271	1.089

Each hydroxyapatite crystal in dentin has 3 zones – internal, external and a hydrated sheath. The research data shows that the hydrated sheath consists of low-molecular Ca-P substances with a composition close to that of octacalcium phosphate (OCP) and dicalcium phosphate dihydrate (DCPD) [15,16]. Teeth devitalization cuts off the blood supply, causing dehydration and temperature changes. This could also be a result of dentinal tubule occlusion with aging. The dehydration disrupts the hydrated sheath and leads to crystallization of OCP and DCPD. Our data show the lowest intensity of hydroxylene bonds in the groups of old and devitalized teeth (G5 and G6). This corresponds to the theory of disruption of the hydrated sheath and water loss. This would potentiate the formation of OCP and DCPD, which presence is confirmed by the available research [15]. The most intensive peaks are in the "young dentin group" (G4). A possible reason could be the fact that the samples were taken from wisdom teeth of young patients, extracted before eruption. The root mineralization process was not completed and the water content was still high.

The peaks corresponding to the phosphate groups - $v_1$and v_3, located at 961 cm^{-1} and between 1015 – 1028 cm^{-1}, respectively, show a decrease in their height in the assessed groups (Fig. 2). The decrease in groups G5 and G6 could be a possible change in the hydroxyapatite due to transparent dentin formation. Transparent dentin is deposited thoroughout the lifetime of the tooth and increases with age [17]. The process begins at the apex and moves coronally. It is believed that its secretion is connected to the death of odotoblast cells (due to apoptosis, hypoxia or tooth deviatalization) [18]. The result is the release of biologically active substances that extract the calcium ions from the peri- and intertubular dentin. Those higher calcium concentrations lead to passive precipitation in the dentinal tubule's lumen and leads to a decrese in hydroxyapatite in peri- and intertubular dentin [19]. This corresponds to our data and the observed decrease in the peaks for modalities $v_1$and v_3. The lower intensity of those peaks for group G4 could be explained with its origins – young wisdom teeth with incomplete mineralization.

The carbonate radical between 860 – 890 cm^{-1} (v_2 mode) is highly pronounced, with good intensity. Therefore, we could state that the root dentin conatins carbonated hydroxypatite.
The absorbant peaks that correspond to the organic components are located between 1200 cm^{-1} and 1600^{-1}. The peaks with the highest intensties are located between 1640 – 1660 cm^{-1} (Amide I), 1525 - 1554 $см^{-1}$ (Amide II), 1241 $см^{-1}$ (Amide III) and 1280 – 1310 $см^{-1}$ (C-N). In the "young dentin group" those peaks have the highest intensity and merge together, which is an indication for and amorphous structure with low crystallinity (Fig. 2). Therefore, some changes have occured in the organic matrix. It can be suggested that with the deposition of transparent dentin the integrity between the hydroxyapatite crystals and the collagen fibrils is disrupted. This leads to instability in the collagen's triple helix and a tendency to denaturation [20]. This could affect the mechanical properties of dentin.

It can be stated that:
1) The hydroxyapatite content in root dentin is decreased, the collagen structure is changed;
2) Aging and endodontic treatment could lead to changes in root dentin that affect both the hydroxyapatite and the collagen structure.

Therefore, endodontic treatment could potentiate mechanisms, similar to those observed during the physiologycal aging of dentin.

The infrared spectroscopy can provide quantitive data through calculation of the peak areas and comparison of their relative intensity (Table 2). The analysis of fig. 3 shows the ratio between the areas of v_1PO_4 and v_3PO_4 (for hydroxyapatite) to Amide I (for collagen). The root dentin groups show changes in both the inorganic and organic components.

7. Conclusion

The changes that occur in dentin as a result of endodontic treatment can accelerate the process of hydroxyapatite dissolution and collagen desintegration. This process is due to mechanisms similar to those observed with ageing. In contrast to aging though, the process is sped up and affects mainly the root dentin.

8. Future Scope

On the limitations of this study was the small number of samples used for the FTIR-ATR analysis. A greater number of samples is required, as well as future investigation of ETT treated with different irrigation solutions, as well as different obturation techniques (cold lateral condensation, single cone technique, warm vertical condensation, hybrid techniques).

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