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

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Abstract: ***Dentin determins the mechanical properties of teeth and distributes the mechanical pressure. The aim of our study was to determine the changes that take place in samples of crown dentin in vital and devitalized teeth using FTIR-ATR. The samples used were taken from young crown dentin, old crown dentin and crown dentin from endodontically treated teeth (ETT). The spectroscopic images obtained showed typical spectres for dentin, with absorbance peaks between 800 – 3500/cm^{-1}. No differences in the dentin composition were observed, regardless of age and endodontic treatment. No presence of new chemical bonds was noted and all of the basic dentin components were detected. The conducted analysis showed that crown dentin consists of hydroxyapatite with good crystallinity, a high concentration of phosphate groups and undamaged organic matrix.***

Keywords: Dentin, FTIR, ATR, spectroscopy

1. Introduction

The mechanical properties of teeth are determined by the structures that comprise them – enamel, dentin, cementum, of which dentin is the most abundant. It distributes the mechanical pressure generated during mastication from enamel towards the jaw bones [1]. Its fracture resistance is vital to the longevity of the tooth and its future prognosis. The factors that can influence and alter the mechanical properties of the remaining dentin are numoerous – from the decrease of hard dental tissues (including the loss of the pulp chamber roof and proximal surfaces) to the subtle chemical changes that might occur in its structure. Those changes could be a result of both endodontic treatment and the natural ageing process.

2. Literature Survey

The clinical investigations on endodontically treated teeth (ETT) show that, compared to vital teeth, they have decreased mechanical properties and are more prone to fractures. Several studies had been conducted, with an aim to determine the possible physical, biomechanical and chemical changes that might occur in ETT. Among the factors that might have a certain effect are the irrigating solutions and long-term medicaments used during treatment. Sodium hypochlorite has a proteolytic action that desintegrates the polypeptide chains of collagen [2]. EDTA is a chelator that binds calcium ions from the hard dental tissues, which can reduce the microhardness of dentin [3].

In dental medicine, infrared spectrocopy has been used for tracing the changes that occur in dentin after treatments with Nd:YAG laser, hydrogen peroxide and sodium hypochlorite [4-6]. The method's high precision and accuracy makes it useful for the investigation of changes in dentin during ageing, as well as after endodontic treatment.

3. Problem Definition

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

4. Methodology/ Approach

4.1 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 °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
Crown dentin	G1	G2	G3

4.2 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.

4.3 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).

5. 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 [5,7]. 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.

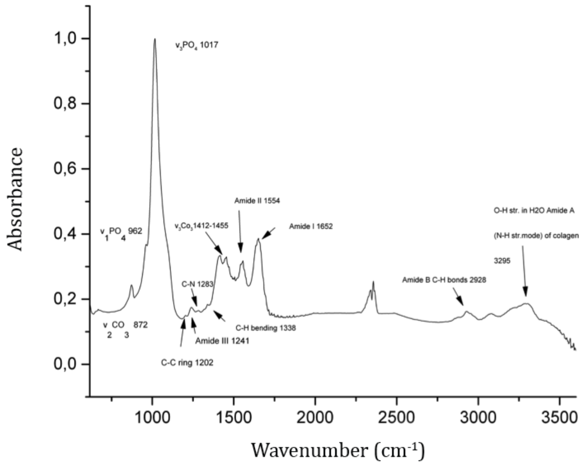

Figure 1: Typical infrared dentin spectre. The absorbance peaks between 800 – 3500/cm^{-1}are marked with arrows.

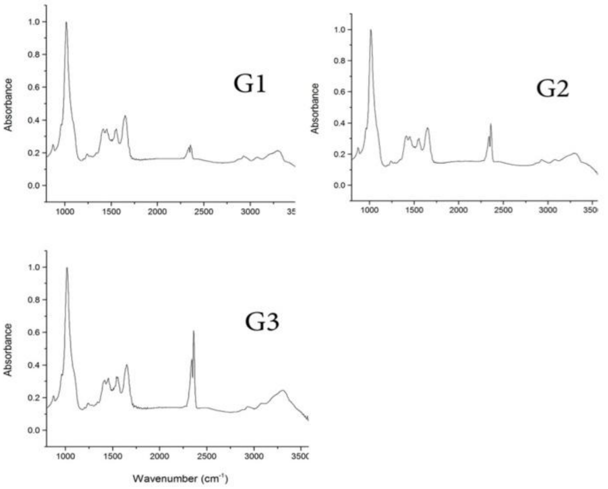

Figure 2: Spectograms of “young crown” dentin (G1), “old crown” dentin (G2), “devitalized crown” dentin (G3).

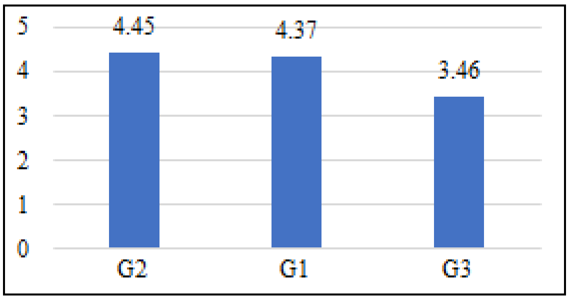

Figure 3: Comparison of “phosphate/amide” groups ratios for all samples (G1 – G3).

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) [8-10].

Collagen is represented by amide (-NHCO-), C-N, C-H, C-C and N-H bonds [11]. Water is found in two states – loosely bonded (absorbed) and strongly bonded (embedded in the structure of dentin). Hydrexylene 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 no pronounced differences between them. Due to the low crystallinity of dentin, the hydroxylene bonds are weal and are hardly visible.

Hydroxyapatite in the dentin structure can be pure ($Ca_{10}(PO_4)_6(OH)_2$) or some of its functional groups can be replaced by a carbonate group. When the hydroxylene group is substituted, it is marked as carbonited hydroxyapatite type A, and when the phosphate group is replaced – as type B. Because of the presence of those derivatives of pure hydroxyapatite, the spectrograms show peaks for the hydroxylene, carbonate and phosphate radicals.

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
G1	1	2.682	32.975	11.531	3.092
	2	4.934	50.184	15.019	5.668
	3	4.938	49.356	12.440	4.364
G2	1	5.198	52.576	13.027	4.435
	2	4.830	54.614	17.654	3.367
	3	6.742	54.759	11.076	5.553
G3	1	3.700	47.856	14.737	3.498
	2	2.557	44.902	14.279	3.323
	3	3.258	45.895	13.816	3.558

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

The phosphate radical has four vibrational modes, two of them - $v_1$and v_3, are visible in infrared spectroscopy. The highest intensity belongs to the v_3 mode. In our study, it is observed in the range between 1015-1028 cm^{-1} in all of the groups. The other mode (v_1PO_4) is located around 961 cm^{-1} and is visible only under great magnification. Those peaks are well manifested with no differences in intensity.

The carbonate radical also has four vibrational modes. Among them, $v_2$and v_3 are the most important, whereas the other two have a very low intensity [12]. Some of them are visible in the range between 1420 – 1540 cm^{-1} and are overlapping with the peaks for the organic components. Between 860 – 890 cm^{-1} the v_2 mode is observed, which is well represented in our study. According to the literature, the peak located at 873 cm^{-1} corresponds to carbonated hydroxypatite type B, and those at 879 cm^{-1} – to type A. Die to their close proximity, a spectrogram with a much higher resolution is needed to make them fully visible. In our results these modes are represented by only one peak, with no opportunity of distinguishing between the two. In all of the spectograms there are no pronounced differences in this area.

The organic matrix consists mostly of collagen and small quantities of lipids, mucopolysaccharides, keratine and proteins [13]. The absorbant peaks that correspond to the organic components are situated between 1200 cm^{-1} and 1600^{-1}. In this area of the spectrograms there is additional overlapping of the peaks that indicate the water and carbonate content. The peak with the highest intensity is located between 1640 – 1660 cm^{-1} and is marked as Amide I. It is representative of the peptide bonds in collagen. Other amide peaks are visible next to that one: Amide II (1525 - 1554 см$^{-1}$), Amide III (1241 см$^{-1}$) and C-N (1280 – 1310 см$^{-1}$). In the crown dentin samples the amide peaks are clearly defined and visible, which is an indication for a sound, highly crystalline structure with no changes or disturbances in the collagen network. 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). There are no statistically important differences between the organic and inorganic components in the groups.

6. Conclusion

The described chemical substances are visible in the spectrograms of all crown dentin groups. No differences in the dentin composition were observed, regardless of age and endodontic treatment. No presence of new chemical bonds was noted and all of the basic dentin components were detected. The conducted analysis showed that crown dentin consists of hydroxyapatite with good crystallinity, a high concentration of phosphate groups and undamaged organic matrix.

7. Future Scope

On of the limitations of this study was that the described analysis covered only crown dentin samples, whereas the fractures of ETT most often include the roots. Those vertical fractures are the ones with the most uncertain prognosis as well, as any future restoration is dubious and hard to achieve. Physiological processes, like transparent dentin formation that occur in root dentin, could also influence the incidence of root fractures. Further reasearch is needed as to whether any changes in the chemical structure of root dentin can be observed as well.

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Author Profile

Ekaterina Karteva, graduated from the Medical University –Plovdiv, Bulgaria in 2013. Started working as an assistant professor in 2014 in the Department of "Operative Dentistry and Endodontics" in the same university. Successfully defended her PhD thesis in December 2016 on the topic of "Restoration of endodontically treated teeth – survey, biomechanical and clinical investigations". Areas of interest include the restoration of endodontically treated teeth, biomechanical properties of dentin, and immune responses to chronic periapical lesions.

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