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Original article

RAMAN SPECTROSCOPY ANALYSIS OF CORONARY DENTIN OF VITAL AND ENDODONTICALLY TREATED TEETH

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ABSTRACT

Purpose: The present study aimed to investigate the properties of coronary dentin of vital and endodontically treated human teeth using Raman Spectroscopy.

Material&Methods: The samples used were taken from coronary dentin of intact extracted human teeth, ultrasonically cleaned and stored in a 0.2% thymol solution at 4 degrees Celsius for up to 3 months. They were divided into two groups – "vital dentin" and "endodontically treated dentin" samples. Each sample was crossed-sectioned, embedded in resin, polished and subjected to Raman spectroscopy with a Horiba Jobin-Yvon T64000 triple-grating spectrometer, using a CdHe laser with 4 ìm diameter of the laser spot and 2.5mW of power on the examined surface. The resultant diagrams were analyzed using the software package Origin 9.6 andunderwent baseline correction and normalization. A comparison between the two studied groups was made.

Results: The results from the "vital dentin" group showed a well-expressed crystalline structure, with clear peaks corresponding to pure and b-carbonated hydroxyapatite. The results of the "endodontically treated dentin" group showed the presence of new chemical compounds – octacalcium phosphate, tricalcium phosphate and dicalcium phosphate dihydrate, as well as increased intensity of the -carbonated hydroxyapatite peaks.

Conclusions: The presence of mineral substances with low calcium content could contribute to a decrease in dentin toughness, which in turn can lessen the mechanical properties of endodontically treated teeth. Raman spectroscopy can be a useful tool in the detection of these dentin components.

Keywords: Raman spectroscopy, endodontically treated teeth, crown dentin,

INTRODUCTION

The ideal method of restoration of endodontically treated teeth has been the subject of a heated debate in dentistry for many years. These teeth are more fragile and prone to fracture than vital teeth and therefore present a clinically relevant problem in contemporary endodontics [4]. The reasons behind this phenomenon are multiple and diverse. For example, the loss of structural integrity due to extensive caries lesions is discussed, as well as fractures and final cavity preparation procedures. Possible modifications in dentin structure, water loss and collagen alteration are also controversial topics. In particular, disruptions in the covalent bonds in collagen that normally ensure dentin's stability and tensile strength have been questioned. Detailed information on these subtle ultrastructural changes is scarce, as more powerful, precise and sophisticated research methods are required to detect them. Raman spectroscopy is a potent non-destructive method with a wide range of applications in the medical field. It provides information about the chemical content of the studied samples, such as biological/biomedical specimens in cancer research, medications in the pharmaceutical industry and, more recently, neuroscience. [1-3] The method utilizes energy from infrared electromagnetic waves in the scope between 300 MHz - 3 GHz. Part of the electromagnetic radiation is absorbed by the molecules of the studied sample. The intensity of this absorption depends on the frequency of the electromagnetic waves and those variations comprise the absorbance spectra of the sample. This provides information about its chemical components and their quantities.

The objective of this study was to investigate the composition changes that occur in coronary dentin of endodontically treated teeth using Raman spectroscopy. Any alteration in dentin's components or structure (with age or endodontic treatment) could give researchers an insight into the problems clinicians face when restoring them [5].

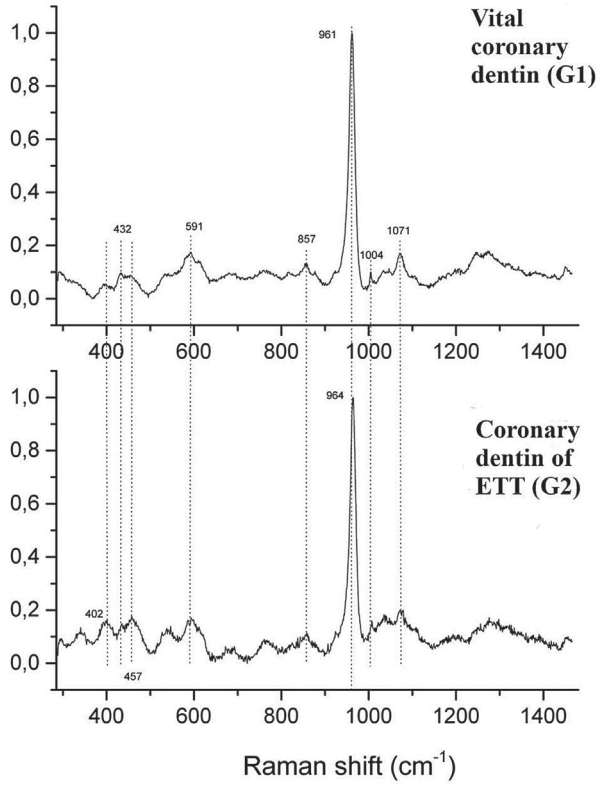
MATERIALS AND METHODS

The samples used were taken from coronary dentin of intact extracted human teeth. They were ultrasonically cleaned and stored in a 0.2% thymol solution at 4 degrees Celsius for up to 3 months. The "vital dentin" samples (G1) were taken from impacted third molars (x6), to ensure the dentin has not been subjected to external irritations and exclude possible tertiary dentin formation. The "endodontically treated dentin" samples (G2) were taken from extracted premolars (x6) that have undergone endodontic treatment. Each sample was cross-sectioned at the tooth equatorwith a diamond separator. The sectioned coronal part was embedded in epoxy resin and polished with a silicone carbide paper of 1200 grit size. Raman spectroscopy was performed with a Horiba Jobin-Yvon T64000 triple-grating spectrometer, using a CdHe laser (IK Kimmon Koha Co. LTD) with 4 ìm diameter of the laser spot and 2.5mW of power on the examined surface. The evaluation of the Raman spectra was performed in the software package Origin 9.6. A representative Raman spectrogram for each studied group was created by taking the average Raman values in the corresponding group and normalizing them to the average intensity of the samples. In addition to the normalization, the presented spectrograms have undergone baseline correction.

RESULTS

The representative resultsare shown on the spectrogram in fig. 1. The spectrograms for the two groups (G1 and G2) are presented in a way that makes their comparison easier.

Fig. 1. Comparison of representative Raman spectrograms of groups G1 and G2.



The μ_1(PO4) stretching corresponds to hydroxyapatite and is the strongest observed peak –near 960 cm^{-1}[6]. The peaks at 402 - 408 $cm^{-1}$and 458 cm^{-1}correspond to the chemical compound tricalcium phosphate(TCP) [7]. Peak 432 cm^{-1} corresponds to the $v_2PO_4^3$ stretching of hydroxyapatite. It is clearly defined in G1, but sharper and with a higher intensity in G2. The peaks in the range between 526-547 cm^{-1} coincide with octacalciumphosphate (OCP). [7] They cannot be discerned in G1, but are much more intensive in G2. Peak 591 cm^{-1} is the $v_4PO_4^{3-}$stretching of hydroxyapatite, clearly defined in G1. In G2, though, there are 2 peaks in that proximity at585 cm^{-1} and 593 cm^{-1}, meaning the peak is fragmented with a dominant stretching at 593 cm^{-1}. This is an indicator of the presence of dicalcium phosphate dihydrate (DCPD). The 1003 cm^{-1} peak coincides with the chemical compound OCP. It has a higher intensity in G2, with a slight displacement to the right (1007 cm^{-1}).

Traces of the organic component of dentin can be found at 857 cm^{-1} – this corresponds to the C-C proline chemical bond. It has a lower intensity in G2. There are almost no definable peaks in the interval between 1034-1039 cm^{-1}in G1. In the endodontically treated teeth group, though, there are numerous peaks with higher intensity, the most prominent of which is at the 1039 cm^{-1}, assigned to TCP [7]. Peak 1071 cm^{-1}corresponds to the $v_1CO_3^{2-}$ stretching of b-carbonated hydroxyapatite and is visible in G1. G2 exhibits numerous, poorly defined peaks in this range (1070-1078 cm^{-1}), which are assigned to several chemical compounds like b-carbonated hydroxyapatite, TCP and hydroxyapatite.

The results from the G1 group show awell expressed crystalline structure, with clear peaks corresponding to pure and b-carbonated hydroxyapatite. The results of the G2 group show the presence of new chemical compounds – OCP, TCP, as well as increased intensity of the b-carbonated hydroxyapatite peaks.

DISCUSSION

The increased amount of chemical compounds with less calcium content in the G2 group can be explained by the process of dehydration and recrystallization that take place in endodontically treated teeth. Research shows that hydroxyapatite crystals in the dentin are covered with a hydrate layer with a composition similar to that of DCPD and OCP. [8-10] It is perceived that this layer is easily destroyed with dentin dehydration. This would lead to an increase in the crystallinity and secondary precipitation of DCPD and OCP. The process of dehydration is accelerated with endodontic treatment. The loss of pulp vitality and the subsequent termination of blood flow, as well as the endodontic treatment procedures that follow lead to a decrease in the water content. [11-13] The effective dehydration coefficient for dentin was estimated at -810 microns/m/(% weight loss). [14] Therefore, these conditions could favour the formation of impurities in the dentin structure.

Moreover, dehydration is considered a predisposing factor for the occurrence of vertical tooth fractures after root canal treatment. [13] This is explained by the development

of residual shrinkage strain in the tooth structure, which leads to the formation of microcracks. Under masticatory pressure, these microcracks can undergo growth and, eventually, lead to tooth fracture.

Research shows that apatite impurities with a low calcium content have decreased mechanical properties. [15] Such impurities are TCP, OCP, DCPD. The Ca/P ratios of stoichiometric OCP and TCP are 1.33 and 1.50, respectively. In contrast, that of hydroxyapatite (HA) is 1.67. [16]

Phosphate ions can form chemical compounds with calcium ions in different Ca/P ratios. The stable phases in an aqueous solution are DCPD and HA. OCP is a metastable component that can transform into either DCPD or HA depending on the environmental conditions and, most notably, their acidity. It is considered a possible precursor of bone and tooth apatite crystals and is likely to form clusters.

OCP is the only compound from the calcium phosphate family that can incorporate carboxylate ions into its crystal lattice and is presumed to be the precursor of biologically formed apatite. Its crystal structure is constructed of a layer of Ca-deficient apatite-like structure and a hydrated layer, similar to that of brushite (or DCPD). This explains the possibility of the incorporation of different ions in its structure. It is characterized by high solubility and biodegradability. [17]

The presence of DCPD and TCP is associated with decreased tension resistance. [18, 19] The increased content of these compounds in endodontically treated teeth could contribute to their reduced toughness, making them more prone to vertical root fractures. Moreover, recent studies have shown that the use of hydroxyapatite-based sealers containing sodium hydroxide and zinc oxide can lead to the formation of new minerals with poor crystallinity. [20] The characteristics of these immature crystallites and how they might alter the mechanical characteristics of endodontically treated teeth require further research in this area. There is emerging evidence that oxipatite-based cements or sealers might contribute to high hydroxyapatite solubility, instability and enhanced remineralizing activity. [21] Therefore, such substances might be unsuitable for use in root canal obturation but prove highly beneficial in biological endodontic treatment.

CONCLUSIONS

The detected presence of àpatite impurities - OCP, TCP and DCPD is a sign of an increased concentration of mineral substances with lesser calcium content in G2. Literature data show that these substances present a decrease in toughness, which in turn can lessen the mechanical properties of endodontically treated teeth. Raman spectroscopy can be a useful tool in the detection of these dentin components, as well as a stepping stone for further research on the amount of water content loss, to help further our understanding of how the loss of tooth vitality affects its prognosis.

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