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SPECTRAL CHARACTERISTICS OF DENTAL HARD TISSUES AND THEIR CLINICAL RELEVANCE

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Abstract

Raman spectroscopy is a molecular specific technique which can be used to develop a fundamental biochemical understanding of tissue physiology and pathology. This method is non-invasive and non-destructive: it utilizes a monochromatic light source to determine sample chemical composition. Raman spectroscopy has become widely used in different fields of medicine, including dentistry. The aim of the study is to assess the spectral characteristics (Raman fluorescent components) of dental hard tissues and to evaluate the usability of the method for the assessment of mineralization of dental hard tissues. In the in vitro study, Raman-fluorescent spectroscopy of teeth (incisors, premolars, molars) extracted due to clinical indications was performed. The results of the preliminary study confirmed the advantages of using wet samples for the evaluation, as it allowed to increase the sensitivity of Raman spectroscopy. Therefore, all the teeth used were kept for 30 minutes in deionized water prior to the experiment. Laser spectroscopic complex "InSpectr M" (Russia) with 514 nm wavelength was used. A qualitative and quantitative assessment of the content and distribution of hydroxyapatite in sound and demineralized dental hard tissues (enamel, dentin, cement) was performed. According to our results, for all groups the content of hydroxyapatite in enamel, as a more mineralized tissue, was higher, than that in dentin, cement and enamel carious lesions. High sensitivity and rapidity of the method as well as the possibility to quantitatively assess the spectroscopic results were revealed. That allows using Raman spectroscopy in the assessment of mineralization and remineralization of dental hard tissues, effectiveness of remineralizing medications and algorithms of their use in clinical practice.

Keywords: *Raman spectroscopy, demineralization, remineralization, hydroxyapatite, enamel, dentin, cement.*

Introduction

Raman spectroscopy is a molecular specific technique that can be used to develop a fundamental biochemical understanding of tissue physiology and pathology. This method is non-invasive and non-destructive: it utilizes a monochromatic light source to determine sample chemical composition. Raman techniques satisfy many of the criteria required for the adoption of a diagnostic technique in clinical practice: sensitivity to changes in tissue, *in vivo* application, and information obtained noninvasively, in real time [*Hanlon E et al., 2000*]. Therefore, Raman fluorescent spectroscopy has become a widely used method in different fields of medicine, including dentistry.

According to the WHO report, dental caries is a serious public-health problem, which impacts individuals and communities in terms of pain and suf-

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fering, impairment of function, and reduced quality of life [*Petersen P, 2008*]. Dental caries is an irreversible Shafer microbial disease that affects calcified structures of the tooth. Despite several preventive measures taken to reduce the incidence of dental caries, improved oral health education, and community water fluoridation, it is still one of the most prevalent diseases experienced by most individuals at some point in their lives. Dental caries has a multifactorial etiological background, such as dental plaque [*Kutsch V, Yong D, 2011*], dietary carbohydrates, saliva, tooth morphology, and pH lowering and cariogenic potential of the dental plaque [*Van Houte J, 1994*]. Clinically, dental caries is characterized by progressive demineralization of inorganic structures and destruction of the organic structure of the tooth [*Sivapathasundaram B, Raghu A, 2006*]. Initially dental caries affects enamel and remains asymptomatic until it reaches the dentin and pulp. By the time patient experiences symptoms, caries clinically shows a large cavity with 30 to 40% demineralization of inorganic matter, this is when it will be radiographically evident. Various techniques used to diagnose caries [*Pretty I, 2006*] detect dental caries mainly in advanced stages, [*Carvalho F et al., 2013*] when prevention can only stop the progression but cannot revert the destruction of tissues. Early detection of dental caries is complicated, as these (incipient) lesions are non-cavitated [*Gomez J et al., 2013*] and appear as white spots, which can be detected visually only when the enamel surface is dry. Therefore, additional methods are essential for early diagnosis and prevention of dental caries.

As stated previously, dental caries is characterized by demineralization of inorganic substance (hydroxyapatite crystals) and destruction of organic substance (collagen matter). Raman spectroscopy allows to characterize hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$). These crystals undergo dissolution in acidic pH: phosphate ions, $(PO_4)_3^-$ and hydroxyl ions (OH^-) react with the hydrogen ions (H^+) in the tooth–biofilm interface and form $H_2PO_4^{2-}$ [*Usha C, Sathyanarayan R, 2009*]. The $(PO_4)_3^-$ forms dissolved crystals, which show several increased Raman peaks at 1043, 590, and 431 cm^{-1} [*Hill W, Petrous V, 2000*]. On the other hand, during the caries process the inorganic matter of the tooth is gradually replaced by organic matter, which shows stronger organic peaks than normally seen [*Alias A et al., 2009*]. These changes can be revealed while studying tissue composition with Raman spectroscopy, in which irradiation excites chemical bonds in the specimen resulting in secondary radiation which is captured by a detector and analyzed [*Ko A et al., 2008*].

Recent studies on Raman spectroscopy have shown promising results in diagnosing early dental caries [*Ko A et al., 2006*]. The study by S. Yang and co-authors showed that it is possible to assess mineral components of tooth tissue using Raman spectroscopy. The authors compared carious areas of enamel with healthy areas of the tooth at wavelengths 960 and 880 *cm*-1 corresponding to $(PO_4)_3^-$, and $(CO_3)_2$, respectively, since these peaks clearly show the difference between healthy and carious areas of the tooth. Ko et al. studied extracted natural teeth with incipient carious lesion using polarized Raman spectroscopy [*Ko A et al., 2006*]. These teeth demonstrated white spots and areas of demineralization with an intact surface, which were not diagnosed by routine caries detection techniques. The authors reported changes in the peak intensities at 1104, 1069, 959, 608, and 590 *cm*−1 as a result of changes in crystal orientation due to the demineralization. Reduced Raman polarization anisotropy was demonstrated at 959 *cm*−1, which refers to phosphate ions within the hydroxyapatite crystals. Their results showed sensitivity of 97% and specificity of 100% in detecting early dental caries. This study also provided ρ959 or A959 as a single numerical value to measure quantitatively the destruction process due to caries. Ko A. and co-authors also developed new fiber optic-coupled polarization-resolved Raman spectroscopic system to simultaneously record orthogonally polarized spectra. They used a combination of multimode optical fibers and polarizing beam splitters and reported a higher depolarization ratio of 959 cm^{-1} peak [*Mohanty B et al., 2013*].

Ionita examined extracted natural teeth with questionable caries and observed reduced anisotropy of Raman spectra due to demineralization by the caries process and increased scattering of light

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due to fluorescence process [*Ionita I, 2009*]. This study examined the fluorescence using a spectrofluorometer. Choo-Smith L. and co-authors attributed this increased red fluorescence to the presence of organic waste in the caries-affected area, such as advanced glycation end products [*Choo-Smith L et al., 2010*].

Kozloff K. and co-authors conducted a comparative study between histological appearance, optical coherence tomography, and Raman spectroscopic findings and concluded that the peaks observed by Raman spectroscopy confirmed the diagnosis of early dental caries [*Kozloff K et al., 2004*]. The conclusion was supported by the findings of histological sections and optical coherence tomography imaging. Alias et al. studied caries-affected natural teeth and categorized extracted teeth as unaffected, affected, and heavily affected according to FT Raman spectroscopy analysis [*Alias A et al., 2009*]. The results of their study showed no significant difference between the three groups; however, they categorized the intensities between the three groups using Wallis and Wilcoxon rank sum tests. The intensity arbitrary unit (au) of the heavily caries-affected enamel was 5528.72, which was much higher than affected (5047.75) and unaffected enamel surfaces (5013.03).

Carvalho F. and co-authors compared the fluorescence value recorded by Raman spectroscopy and an advanced caries detection aid (Diagnodent, Lake Zurich, Illinois). The fluorescence readings of the enamel caries, dentin caries, and sound dental structure were measured and the values were compared with the Raman spectroscopy readings. The study showed a significant difference in the fluorescent readings between enamel caries (16.4±2.3) and dentin caries (57.6±23.7). They noticed higher fluorescence values in the samples with low Raman peaks of phosphate (960 cm−1) and fluoridated apatite (575 *cm*−1); the change in fluorescence values was attributed to the higher bacterial metabolic activity and organic content. This study also compared the sensitivity of Diagnodent and Raman spectroscopy in diagnosing dental caries. The study concluded that Raman spectroscopy can serve as valuable tool in diagnosing early dental caries and Diagnodent readings can be used to diagnose advanced stages when there is extreme demineralization and higher organic matter instead of inorganic matter [*Carvalho F et al., 2013*].

Choo-Smith et al. employed polarized Raman spectroscopy (PRS) in conjunction with optical coherence tomography to detect early dental caries and used polarized Raman spectroscopy to prevent false-positive results [*Choo-Smith L et al., 2010*]. They derived an optical attenuation coefficient parameter from high-resolution images of optical coherence tomography and differentiated caries-affected and unaffected teeth. In addition to optical coherence tomography, the Raman depolarization ratio of the phosphate vibration at 959 *cm*−1 measured from parallel and cross-polarized Raman spectroscopy provided valuable information to diagnose early dental caries with high specificity and sensitivity. Similar research conducted by Prabhakar N. and co-authors also proved Raman spectroscopy as a valuable modern tool [*Prabhakar N et al., 2011*].

The aim of the present study was to assess the spectral characteristics (Raman and fluorescent components) of dental hard tissues and to evaluate the suitability of the Raman-fluorescent spectroscopy method for the assessment of dental hard tissues mineralization.

Materials and methods

Fluorescent Raman spectroscopy of permanent teeth hard tissues (incisors, premolars and molars), extracted according to clinical indications was performed. Laser spectroscopic complex “EnSpectr M” (Russia) with 514 *nm* wavelength was used (Fig. 1).

Figure 1. *Spectrometer “EnSpectr M”.*

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Raman spectrometer "EnSpectr M" measures Raman spectra or photoluminescence of fluids, powders, gels and solid objects. It consists of source of laser radiation, and the complex for detection, filtration and analysis of resulting radiation. Spectrometer is equipped with low-noise multichannel detector-analyzer (CCD-scale) for measuring amplitude and spectral characteristics of scattered radiation. The spectral range includes areas of molecular vibrations from organic and inorganic substances and provides a real-time (within several seconds) measurement of Raman and fluorescent spectra of the explored sample.

Insert No 1 is used to assess liquids or powders in glass tube (through the glass wall).

Insert No 2 is used to assess solid objects of small sizes (pills, crystals and others, including teeth). The sample is fixed in the cap opposite to the aperture of the spectrometer and then the cap is screwed on the spectrometer tightly.

Software of this spectrometer allows to perform qualitative and quantitative analysis of acquired spectra by comparing them with the reference spectra from the database (the method described by Alexandrov and Kukushkin).

Thirty teeth were used in the study (10 premolars, 10 incisors, 10 molars) extracted according to clinical indications. Preliminary measurements were accomplished of wet and dry teeth, which showed better reproducibility of the measurements for hydrated samples compared with dry samples. It has been also shown that Raman intensity values were 30-50% higher for the hydrated teeth compared with the dry teeth.

For the qualitative assessment of Raman radiation intensity (in relative units) the maximum and minimum values of the peak were noted as well as the intensity of fluorescence. The difference between maximum and minimum peak height divided by the intensity of fluorescence was counted (Raman intensity).

In a separate session, the Raman intensity of enamel of 10 incisors was assessed before and after the application of 10% solution hydroxyapatite.

Table 1.

Spectral Raman characteristics of intact dental hard tissues

Dental hard tissue N=10	Peak maximum		Peak minimum		Raman spectrum	
	y-peak maximal intensity, relative units	x - peak wavelength, λ, $см^{-1}$	y – peak minimal intensity, relative units	x- peak wavelength, λ, $см^{-1}$	Y - peak Raman intensity, relative units	x- peak wavelength, λ, $см^{-1}$
Incisor						
Enamel	11134 ± 40	963	8079 ± 43	963	3058 ± 40	963
Caries of enamel	23819 ± 60	963	22949 ± 50	963	870 ± 056	963
Dentin	15276 ± 65	963	14282 ± 46	963	994 ± 50	963
Cement	4662 ± 71	963	3967 ± 62	963	695 ± 67	963
Premolar						
Enamel	12690 ± 42	963	11647 ± 67	963	1043 ± 060	963
Caries of enamel	14906 ± 86	963	14001 ± 56	963	905 ± 48	963
Dentin	12033 ± 38	963	10305 ± 73	963	670 ± 64	963
Cement	16270 ± 72	963	15964 ± 29	963	306 ± 40	963
Molar						
Enamel	17637 ± 48	963	16480 ± 70	963	1157 ± 71	963
Caries of enamel	7839 ± 49	963	7742 ± 53	963	97 ± 4	963
Dentin	18081 ± 72	963	17603 ± 70	963	479 ± 70	963
Cement	11813 ± 40	963	11686 ± 43	963	127 ± 4	963

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Results and discussion

The results obtained for the different teeth groups (incisors, premolars, molars) are shown in the table 1.

According to our results, for the incisor group the content of HAP in enamel, as a more mineralized tissue, is higher than that in dentin, cement and enamel carious lesion, comprising 3058-994-695-870 relative units, respectively. The fluorescence intensity showed similar pattern and comprised 22949-14282-8079 relative units for caries, dentin and enamel, respectively, in accordance with organic content of the examined tissues (Fig. 2).

Similar results were obtained for the premolar group: the content of HAP in enamel, as a more mineralized tissue, was higher as compared with dentin, cement and enamel carious lesions, comprising 1043-670-306 and 905 relative units, respectively. The fluorescence of organic substances in dentin was higher (however the differences with caries of enamel were insignificant) and comprised 15964-14001-11647-10305 for caries, enamel and dentin, respectively.

In the molar group the content of HAP in enamel was also significantly higher, than in dentin, cement and enamel carious lesion, comprising 1157-479-127-97 relative units, respectively. The fluorescence values require additional investigation due to controversy in the results (Table 1).

The results shown in the table 2 confirm the advantages of using wet samples for the evaluation, as it allows to increase the sensitivity of Raman

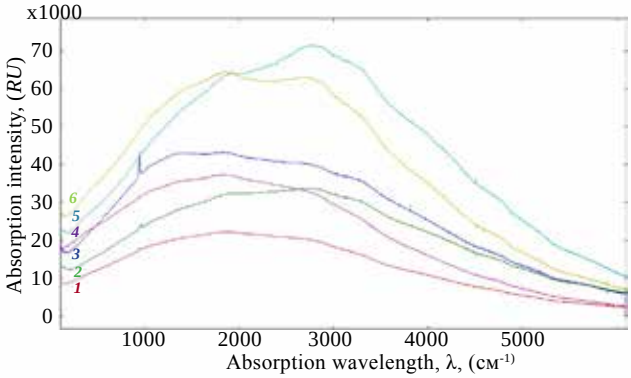

***Figure 2**. The registration of HAP spectrum and spectrum of hydroxyapatite in teeth structures*
Denotations of the curves: (4) enamel cutter, (2) enamel caries cutter, (1) dentine cutter, (5) dentine caries cutter, (3) cement cutter, (6) cement caries cutter.

spectroscopy. The values of Raman intensity comprised 702 and 1520 relative units for dry and wet hydroxyapatite, respectively. Therefore, all the teeth used were kept for 30 minutes in deionized water prior to the experiment (Table 2).

The most interesting results were obtained when assessing the remineralization of enamel. It was shown that after the application of remineralizing solution the content of HAP in enamel increased 2 times (from 849 to 1679 relative units) (Table 2). This finding is important not only from scientific, but also from clinical perspective.

Raman spectroscopy is the next step in the development of fluorescent techniques [*Alexandrov M, Margaryan E, 2017; Alexandrov M et al., 2017; 2018*]. The advantages of this method for the field of medicine including dentistry are: objectivity, on-line real-time measurement, high spatial resolution (around 1Å) sensitivity and reproducibility, small standard error,

Table 2.

Spectral characteristics of wet and dry HAP (synthetic calcium hydroxyapatite powder) and enamel before and after HAP application

Dental hard tissue N=10	Peak maximum		Peak minimum		Raman spectrum	
	y-peak maximal intensity, relative units	x - peak wavelength, λ, *см*$^{-1}$	y – peak minimal intensity, relative units	x- peak wavelength, λ, *см*$^{-1}$	Y - peak Raman intensity, relative units	x- peak wavelength, λ, *см*$^{-1}$
Characteristics of hydroxyapatite						
HAP dry	3326 ± 50	963	2625 ± 50	963	702 ± 50	963
HAP wet		963	2040 ± 40	963	1520 ±39	963
Characteristics of enamel after the application of hydroxyapatite						
Before	43330 ± 60	963	42481 ± 050	963	849 ± 50	963
After	62306 ± 76	963	60627 ± 81	963	1679 ± 71	963

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the use of micro-volumes of the explored substances, small size and portability of the equipment.

Our studies revealed the possibility to recognize organic molecules according to their inelastic scattered radiation spectra [*Sheng R et al., 1991; Kneipp K et al., 2002*]. Raman spectroscopy may be used for reliable assessment of mineralization and demineralization of normal hard tooth tissues and various lesions [*Tsuda H, Arends J, 1997; Traminia P et al., 2000; Alexandrov M, 2008; Ramakrishnaiah R et al., 2015; Timchenko E et al., 2016*]. Therefore, this diagnostic method may be widely used for the assessment of hard tooth tissues mineralization, efficacy of remineralizing therapy and it is capable of increasing the effectiveness of preventive dental care.

Raman spectroscopy method is sensitive and allows for reliable real-time assessment of mineralization of both normal and decayed dental hard tissues.

CONCLUSION

Raman spectroscopy method is sensitive and allows for reliable real-time assessment of mineralization of both normal and decayed dental hard tissues.

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