Chemometric analysis of Raman spectra of tissues of the teeth with periapical periodontitis

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Abstract. The Raman spectra of dental tissues with periapical periodontitis have been analyzed. Chemometric analysis of the Raman spectra of hard dental tissues of healthy patients and the patients with periapical periodontitis has been carried out. The main spectral features of dental tissues with periapical periodontitis have been identified, which will further allow developing new methods of early detection of periapical periodontitis.

1. Introduction
Periapical periodontitis is a widespread dental disease that affects 5-15% of the population of all countries according to different sources [1]. Its delayed treatment can cause bad cases of periapical periodontitis and further tooth loss.

Periapical periodontitis is inflammatory disease that affects connective tissue, located between the wall of dental alveolus and tooth root. The disease disrupts periodontium integrity, which causes disruption of its functions such as maintaining teeth in dental alveolus and uniform distribution of pressure on teeth during mechanical loads [2].

The pathological process in one type of tissues of dental apparatus can also extend over neighboring tissues. E.g., progressive periapical periodontitis causes crack lines on tooth cementum surface with depth depending on severity of the disease [3].

The Raman spectroscopy method allowing receiving high-resolution spectra of subjects noninvasively and without preparing is currently widely used among the promising optical methods of analysis. This method is used in dentistry for studying the features of dental tissues and their changes during pathological processes [4,5], as well as for studying dental biomaterials [6].

The aim of this study is revealing spectral features of hard dental tissues with periapical periodontitis.

2. Materials and methods
The materials of the study were the samples of hard dental tissues, taken from different parts of teeth: I – dentin cross section, II – cross section of root dentin, III – exposed cementum, IV – cementum under the gum, V – enamel. The teeth were harvested from two groups of people: patients with periapical periodontitis (1) and healthy control group (2) (the teeth removed for orthodontical reasons).

Spectral characteristics of samples of hard dental tissue were studied using experimental stand, implementing Raman spectroscopy method, and including fiber-optic probe for Raman spectroscopy RPB-785, laser module LuxxMasterRamanBoxx (λ=785 nm), high resolution digital spectrometer
Andor Shamrocksr303i with the build-in cooling camera DV420A-OE. The experimental stand arrangement is shown in Figure 1.

The Raman spectra were processed using the software Wolfram Mathematica 8. The approximating line (fifth order polynomial) of the autofluorescent component that was further subtracted to receive the Raman spectrum in the background of auto fluorescence was determined using iteration algorithm in the range of 800-2100 cm\(^{-1}\). The received Raman spectra were cleaned up from noises by the smoothing median filter (5 points).

The nonlinear regressive analysis of spectra was made in the software MagicPlotPro 2.7.2 using the method of spectral contour selection and Gauss function deconvolution to isolate the spectral lines and for more detailed analysis.

The results received during the research were analyzed using the software SPSS Statistics 19. The chemometric analysis of the results was made using the Wilks method that is a step-by-step process, during which the Wilks coefficient (\(\lambda\)) is minimized after including every new predictor in regression equation.

Figure 1. Experimental stand: 1 – subject; 2 - Raman probe RPB785; 3 – laser module LuxxMasterRamanBoxx; 4 – laser module power source; 5 – spectrometer Shamrocksr303i; 6 – build-in cooling camera DV420A-OE; 7 – computer; 8, 9, 10 – command cables; 11 – optical fibre; 12 – receiving optical fiber.

3. Results

The main spectral differences of teeth with periapical periodontitis are observed on the line of 956 cm\(^{-1}\) (PO\(_4\)^{3-}), related to mineral components of dental tissues, as well as on the lines of 1230-1270 cm\(^{-1}\) (Amide III), 1665 cm\(^{-1}\) (Amide I), related to organic components of dental tissues.

These changes are caused by destruction of collagen fibers in case of periapical periodontitis and reduction of mineral components during the illness.

For more detailed analysis the further chemometric analysis of received data was made using the principal component analysis (PCA) that allows reducing the dimension of the data, highlighting and visualizing the relevant information.

This method was implemented in the software The Unscrambler X. The used PCA algorithm is described in the work [“Multivariate calibration” by Martens & Næs (Wiley 1991, ISBN 0471930474)]. The results of PCA are shown as data sets: the chart of scores (Figure 2), the chart of loadings (Figure 3).
Figure 2. The chart of scores of the samples, divided into healthy and pathological groups of PCA.

Figure 2 shows the analysis of relationships of groups of subjects based on having dentin pathology. It is shown that the main differences between the two groups of samples are described by the principal component PC-1. A sampling is 113 Raman spectra (46 healthy, 67 pathological). The positive values of PC-1 characterize mainly the Raman spectra of the second group of dentine samples, and vice versa, the negative values characterize the first group of samples. The areas of the groups have an intersection that influences the percentage of correctly classified subjects.

To a lesser extent the dispersion is described by the principal component PC-2, which negative values characterize the healthy group of samples and where the intensities of the Raman lines of 937 (C-C valence fluctuations of collagen type I, corresponding to proline) and 1069 cm\(^{-1}\) (C-O planar valence fluctuations of hydroxyapatite carbonate ion CO\(_3^{2-}\) (\(v_1\))) are prevalent compared to pathological samples. And vice versa the Raman lines of 1555 (C-N valence fluctuation of amide II) and 1739 cm\(^{-1}\) (C=O fluctuations of phospholipids) are prevalent in pathological samples compared to healthy samples. The data areas are well divided except for the part of data in the second quadrant, which is because the data of enamel samples, which are less mineralized than the other parts of teeth, gather in this area.

Figure 3. The chart of PCA loadings.
The analysis of figures 2 and 3 allows concluding: the most meaningful differences between the groups of samples are described by the Raman lines shown in figure 3 and having the highest value of PC – 1 function on module. I.e. the greatest contribution to the principal component for dispersion explanation is from the Raman line 956 cm$^{-1}$, corresponding to P-O symmetrical valence fluctuation PO$_4^{3-}$(v$_1$) of hydroxyapatite. The intensity of the line 956 cm$^{-1}$ is higher in healthy samples than in pathological ones.

**Figure 4.** The chart of PCA scores for the samples, the division of the groups into the areas.

The analysis of relationship between the groups of subjects on the basis of belonging to certain tooth area is shown in Figure 4. It is seen that the dispersion between the groups of samples is mostly described by PC-1 (95%). The negative values of PC-1 mostly characterize the group of Raman spectra of enamel samples, and vice versa, the positive values characterize the group of samples of dentin and cement. The areas of enamel group have an intersection with the other areas, therefore it will be rather difficult to accurately classify the enamel when measuring the Raman spectrum using PCA. The areas of dentin and cement also have a large intersection, but it is seen that on average the line of 956 cm$^{-1}$ of dentin samples has lesser intensity in the Raman spectra than in the group of samples of cement.

4. Conclusion

The chemometric analysis of Raman spectra of hard dental tissues of healthy patients and patients with periapical periodontitis was made. Spectral changes of hard dental tissues taken from people having periapical periodontitis that are seen in the lines of 937 cm$^{-1}$, 956 cm$^{-1}$, 1069 cm$^{-1}$, 1230-1270 cm$^{-1}$, 1555 cm$^{-1}$, 1665 cm$^{-1}$ and 1739 cm$^{-1}$ were revealed as a result of the study. These changes indicate the destruction of hard dental tissues: collagen matrix and the salts of hydroxyapatite.

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