The raman spectroscopy method for evaluation of structural changes in hard tissues of teeth after in-office whitening

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Abstract. Start The experiments in evaluation of hard tissues using the Raman spectroscopy method have been made. Spectral differences between the tooth enamel and dentin before and after the in-office whitening procedure were found as a result of the work. It was shown with the use of the Raman spectroscopy method that the whitening process causes changes of tooth enamel and dentin related to the changes of organic and mineral components.

1. Introduction
The treatment of tooth discoloration is a topical issue in modern aesthetic dentistry. One of non-invasive methods is the whitening of teeth. However, in addition to the positive effects this procedure is accompanied with the changes of mineral composition of oral cavity, structural organization of enamel of teeth, dynamics of pulp microcirculation and hypersensitivity of teeth [1,2].

For example, free radicals of hydrogen peroxide, which is a part of whitening gel, from enamel penetrate to dentin and cause the disruption of the structure of collagen protein molecule, which is 95 % of dentin protein, and according to some research peroxide compounds cause the changes of protein molecule organization in the first ten minutes after the contact of the whitening material with the tissues of teeth [3].

The results of our research trigger the further studying of the influence of hydrogen peroxide on the structure of tooth tissues keeping topical the issue of rapid method of evaluation of hard tissues of teeth after the whitening procedure.

One of the most effective methods of evaluation of tooth structure is the Raman spectroscopy method. This method is similar to the Fourier Transform Infrared Spectroscopy (FTIR) [4]. The Raman spectroscopy is non-invasive, informative and widely used in medicine [5,6].

The works [7, 8] show the research of the structure of teeth, give qualitative assessment of mineral composition and provide morphology of micro relief of hard tissues of teeth with different pathologies using the Raman spectroscopy.

But the existing works of other authors do not show what changes take place in organic structure of hard tissues of teeth after the in-office whitening procedure.

The aim of this work is evaluation of the structural changes of hard tissues of teeth after the in-office whitening using the Raman spectroscopy method.
2. Materials and Methods

The teeth removed for orthodontical reasons were the subjects of the study. The photos of the teeth are shown in Figure 1. All the samples were divided in two groups: the group а – enamel and dentin of the teeth before the in-office whitening procedure, the group b – enamel and dentin of the teeth after the in-office whitening.

The chemical method of Opalescence Xtra BOOST system with 40 % of hydrogen peroxide was used for the teeth whitening. It includes two syringes of "syringe inside syringe" design that blend right before use. One syringe contains 40 % of hydrogen peroxide, the other one contains chemical activator, sodium fluoride and potassium nitrate that reduce the sensitivity of teeth during and after the in-office whitening procedure.

The main method of analysis of the influence of the new whitening method on the structure of teeth was the Raman spectroscopy method implemented by the experimental stand that included RamanprobeRPB-785 (focal length of 7.5 mm), combined with the laser module LuxxMaster LML-785.0RB-04 (power up to 500 mW, wavelength of 784.7 ± 0.05 nm) and the high-resolution digital spectrometer Shamrock sr-303i providing spectral resolution of 0.15 nm with the build in cooling camera DV420A-OE (spectral range of 200-1200 nm) [9].

![Figure 1. Tooth enamel and dentin: I) before the in-office whitening procedure; II) after the in-office whitening procedure](image)

To make the spectra more informative the method of spectral contour modeling was implemented in the software MagicPlotPro 2.7.2, where decomposition of spectrum on the lines described by the Gaussian function was made. The results received during the study were analyzed in the software SPSS Statistics 19 and the chemometric analysis of the results was made using the Wilks method [10].

3. Analysis

Figure 2 shows the average Raman spectra of enamel and dentin for the two studied groups of samples.
Figure 2. The average spectra of enamel (I) and dentin before and after the whitening (II): a – before the whitening, b – after the whitening

Figure 2 shows that significant changes are in the lines related to organic components: 813 cm\(^{-1}\) (C–C stretching (collagen assignment), phosphodiester bands in RNA), 852 cm\(^{-1}\) (proline), 877 cm\(^{-1}\) (hydroxylproline), 1000 cm\(^{-1}\) and 1030 cm\(^{-1}\) (phenylalanine). The line of 1152 cm\(^{-1}\) (proline) also appears in the Raman spectra of dentin of teeth after the whitening. Spectral changes related to the change of organic components of teeth are caused by structural changes of collagen which is part of enamel and dentin. These changes cause the oxidation of collagen matrix that is a result of chemical reaction between chromogen peroxide and amino acid residue with the formation of Schiff base, disulphide, cysteine sulfinic, cysteine sulfonic, cysteine sulfonic acids [11].

After the process of whitening spectral changes also occur in the lines corresponding to mineral components: 956 cm\(^{-1}\) (PO\(_4\)^{3–}(\(\nu_1\)) (P-O symmetric stretch)) and 1071 cm\(^{-1}\) (tyrosine (collagen type I)).

For detailed evaluation of the changes of mineral compound presented by the most intensive symmetrical valence fluctuation \(\nu_1\) PO\(_4\)\(^{3–}\) (~956 cm\(^{-1}\)) and carbonate substituted hydroxyapatite: valence\(^{1}\) B-type carbonate ions, substituted phosphate ions in the apatite grid (1065-1071 cm\(^{-1}\)) [8], and the organic compound nonlinear regressive analysis of the spectra was made including their spectral line decomposition. Figure 3 shows the results of decomposition of spectral contour on the sum of distribution of the Gaussian lines.
The average value of the coefficient of determination of the result spectrum on the initial one in the range of 800-1780 cm\(^{-1}\) was \(R^2 = 0.99\), the relative error of spectral line intensity evaluation \(a\) was less than 6\%, the average standard deviation of the coordinate of the line \(x_0\) was 0.8 cm\(^{-1}\), the average standard deviation of the width of the Gaussian line (HWHM) \(dx\) was 1.8 cm\(^{-1}\).

For relative quantitative analysis of component composition the ratios of the Raman lines intensities to Amide I line intensity were used.

**Figure 3.** Spectral contour decomposition of the researched samples

**Figure 4.** The chart of values of linear discriminant function
The method of linear discriminant analysis in the software IBM SPSS Statistics was chosen for further analysis of spectral lines received after decomposition.

It shows that the most dispersion between the researched groups of samples is described by the function LD-1 (79.5 %). The whole sample consists of 28 Raman spectra. The discriminant function LD-2 describes the 14.8 % of dispersion and does not have clear physical meaning.

The positive values of LD-1 mostly characterize the Raman spectra of enamel samples and vice versa the negative values characterize the Raman spectra of dentin samples. The areas of the groups do not have intersections. The function LD-1 has physical meaning of difference of spectral composition between the whitened and non-whitened tissues of teeth. Furthermore, the non-whitened samples of dentin have lower values of LD-1 than the whitened samples, but the samples of enamel show the reverse trend.

Table 1. Values of the coefficients of the factor structure

| Raman lines | LD-1 | LD-2 |
|-------------|------|------|
| k1308       | 0.022| 0.073|
| k813        | 0.025| -0.044|
| k1152       | 0.034| -0.079|
| k1239       | 0.038| 0.054|
| k852        | 0.049| -0.174|
| k1748       | 0.054| 0.202|
| k1128       | 0.061| 0.162|
| k1203       | 0.061| 0.161|
| k1271       | 0.064| 0.052|
| k1176       | 0.065| 0.127|
| k918        | 0.087| 0.028|
| k877        | 0.093| -0.081|
| k1416       | 0.106| 0.291|
| k1092       | 0.117| 0.201|
| k1000       | 0.126| 0.041|
| k939        | 0.131| 0.124|
| k984        | 0.134| 0.179|
| k956        | 0.145| 0.258|
| k1071       | 0.146| 0.096|
| k1030       | 0.155| 0.246|
| k1054       | 0.160| 0.230|

Figure 4 and Table 1 show that the difference between the tissues of whitened and non-whitened teeth can be found by the function LD-1.

It can be seen that the ratios of organic components to mineral components in the tissues of whitened teeth reduce.

4. Conclusions
Spectrum deconvolution using the method of spectral contour selection and Gauss function deconvolution was made as a result of the study, which allowed under taking expanded component qualitative and quantitative analysis of enamel and dentin of the teeth after the in-office whitening
procedure. Spectral changes of enamel and dentin after the teeth whitening were found in the lines of 956 cm\(^{-1}\) (\(PO_4^{3-}(\nu_2)\) (P-Osymmetric stretch)), 1000 cm\(^{-1}\) and 1030 cm\(^{-1}\) (phenylalanine), 852 cm\(^{-1}\) (proline), 877 cm\(^{-1}\) (hydroxyproline), 1152 cm\(^{-1}\) (proline).

It was shown that the process of in-office whitening causes structural changes of enamel and dentin related to reduction of organic components of teeth compared to mineral components, which is caused by oxidation of collagen matrix during the whitening process.

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