Possibility of Raman spectroscopy method use for evaluation of periodontitis-affected tooth enamel before and after open curettage

P E Timchenko¹, E V Timchenko¹, I V Bazhutova², O O Frolov¹, L T Volova², A Y Ionov¹

¹ Samara National Research University, Samara
² Samara State Medical University, Samara

E-mail: laser-optics.timchenko@mail.ru

Abstract. The results of the research of enamel of periodontitis-affected teeth before and after open curettage are presented in the work. The Raman spectroscopy method was used as an evaluation method of curettage influence on tooth enamel. Chemometric analysis of the Raman spectra of tooth enamel before and after curettage was made. Spectral changes of tooth enamel were detected. It was found that after the procedure of open curettage spectral changes occur in tooth enamel related to the changes in mineral composition and organic matrix forming.

1. Introduction

Periodontitis is one of the most insidious and frequent dental diseases. It develops gradually and in the early stages does not cause any pain or severe discomfort. According to WHO (2002) about 95% of the world adult population and 80% of the child population have periodontitis in some form. The high rate of periodontal diseases is among people of age 20 – 44 (over 65 – 95 %) and of age 15 – 19 (over 55 – 89 %) according to the report of the WHO’s Scientific Group. Late treatment can cause severe forms of periodontitis and subsequently tooth loss [1]. The most common reasons of the disease are wrong or irregular oral cavity care, poor blood supply of gums, nutritional deficiencies. There are a lot of methods of the treatment but the main procedure in the comprehensive treatment is [2] closed and open curettage. The closed curettage of periodontal pocket is used if it is less than 4-5 mm and there are no bone pockets. This procedure includes dental calcareous deposit removing, smoothing out the root and applying medication. The open curettage of periodontal pocket is used when it is more than 4-5 mm or if there are bone pockets. This procedure includes dissecting the gum and further turning the flap opening a clear view of the problem area to the doctor and allowing it thorough treatment. After the treatment the stitches are done [3].

Currently there is no unambiguous information in the literature about the impact of curettage on the structure of tooth tissue.

There are lots of methods of evaluation of the condition of teeth affected by periodontitis, such as orthopantomography, radiography, CAT scan etc. [4]. However, these methods do not allow evaluating the composition and the changes of the structure of teeth after curettage in order to additionally prescribe treatment and regenerative therapies for the best result and long-term preservation of the teeth.
This task can be solved with the use of the Raman spectroscopy method widely used in medicine [5,6]. This method allows evaluating relative composition of biosubjects.

The work [5] shows the changes of the structure of cementum and of mineral composition in case of periodontium disease. The authors compare the composition of the teeth affected by gingivitis, chronic and aggressive periodontitis. The work reveals that in case of chronic periodontitis the mineral composition is higher than in case of gingivitis.

Our previously published work [7] includes the results of spectral study of the tissues of teeth affected by periodontitis. The criteria of early diagnosis of this disease were introduced.

The aim of this work is the use of the Raman spectroscopy method for evaluation of enamel of periodontitis-affected teeth before and after open curettage.

2. Materials and methods
The teeth removed due to chronic periodontitis were the subjects of the study. The periodontitis diagnosis was made clinically and after analysis of the cone beam computed tomography (CT) (the code of the disease according to ICD-10 (1997) – K05.3). The samples were divided in 2 groups: 1st group – the teeth removed from patients before curettage; 2nd group - the teeth removed from the same patients after curettage.

![Figure 1. The subjects of the study](image)

The Raman spectroscopy method was implemented by the experimental stand (Figure 2), that included Raman probe PBL 785 combined with the laser module LuxxMasterLML-785.0RB-04 (power up to 500 mW, wavelength of 784.7 ± 0.05 nm) and high resolution digital spectrometer Shamrocksr-303i providing spectral resolution of 0.15 nm with build-in cooling camera DV-420A- OE [8]

![Figure 2. Experimental stand](image)
The detailed analysis of the Raman spectra was made using the software MagicPlotPro, and with the use of the method of linear discriminant analysis (LDA) in the software IBM SPSS Statistics [9].

3. Results
Figure 3 shows the average Raman spectra of the samples of periodontitis-affected teeth before and after curettage.

![Figure 3. The comparison of the spectra of tooth enamel: 1 – before curettage, 2 – after curettage](image)

The Figure shows that after the open curettage procedure spectral changes occur in enamel, which is related to the change of relative intensity of the lines of 956 cm\(^{-1}\) (\(v_1\) P–O symmetric stretch (PO\(_4^{3-}\)) and 1070 cm\(^{-1}\) (C–O in plane stretch (CO\(_3^{3-}\)v1)). These lines are related to the change of mineral composition. The changes of intensity of the lines of 1449 cm\(^{-1}\) (Lipids and proteins), 1036 cm\(^{-1}\) (phenylalanine (collagen assignment)), 854 cm\(^{-1}\) (Hydroxyproline v(C–C) stretch), related to forming of organic matrix, can also be seen.

The spectral changes related to the change of mineral components after open curettage are caused by the fact that calculus is been removed in the process of open curettage (the process of calculus forming is accompanied by enamel hypomineralization), thus causing the reduction of enamel mineralization [5].

The spectral changes related to the change of organic composition are probably caused by collagen restoring after this procedure.

To make the received Raman spectra more informative nonlinear regressive analysis of the spectra was made including their spectral line decomposition. Figure 4 shows the results of decomposition of spectral contour on the sum of distribution of the Gaussian lines.
Figure 4. Spectral contour decomposition of the samples of enamel

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 $R^2 = 0.999$, the relative error of spectral line intensity evaluation $\alpha$ was less than 5%, the average standard deviation of the coordinate of the line $x_0$ was 0.1 cm$^{-1}$, the average standard deviation of the width of the Gaussian line (HWHM) $\alpha x$ was 1.8 cm$^{-1}$.

For relative quantitative analysis of component composition of hydroxyapatite the absolute values of intensities of the Raman lines of normalized spectra were used.

The method of linear discriminant analysis in the software IBM SPSS Statistics was chosen for further analysis of spectral lines of the researched subjects received after decomposition.

Figure 5 shows the results of LDA comparing of the two groups of samples. 30 spectra of enamel were analyzed. The discriminant function LD-1 describes the 100% of dispersion. The positive values
of LD-1 characterize the Raman spectra of the enamel before curettage. The areas of the groups do not have intersections. Various combinations of parameters were analyzed and 7 lines were identified.

Figure 6. The values of factor structure coefficients.

Figure 6 shows the relevant coefficients of the factor structure matrix that have physical meaning of correlation between variables in the model and discriminating function. The higher the modulus value of LD-1 for variable, the more it defines the difference in the discriminant model between the groups of samples.

The discrimination adequacy of the method is characterized by the value of AUC = 1, that indicates the great quality of the diagnostic tool. The standard error SE was 0%. The optimal cut-off point of the presented algorithm determined according to the balance between sensitivity and specificity was 0.824. The sensitivity and specificity of the diagnostic model in this cut-off point were 100%.

4. Results and discussion
The chemometric analysis of the Raman spectra of teeth enamel before and after curettage was made as a result of the study. Spectral changes of tooth enamel were revealed. It was found that after the procedure of open curettage spectral changes in tooth enamel related to the changes of relative intensity of the lines of 957 cm\(^{-1}\) (v1 P–O symmetric stretch (PO\(_4^{3-}\)) and 1070 cm\(^{-1}\) (C–O in plane stretch (CO\(_2^{3\nu 1}\))) occur. These lines are related to the change of mineral composition. There are also changes of intensity of the lines of 1449 cm\(^{-1}\) (Lipids and proteins), 1036 cm\(^{-1}\) (phenylalanine(collagen assignment)), 854 cm\(^{-1}\) (Hydroxyproline v(C–C) stretch) related to the forming of organic matrix.

These spectral changes indicate the restoring of the tooth enamel tissues after this procedure.

The Raman spectroscopy method can be further used for evaluation of tooth tissues after curettage in dental practice.

References
[1] Yanushevich О О, Kuzmina E M, Maksimovsky Y M, Maly A Y, Dmitrieva L A, Revazova Z E, Pochtarenko V A, Ektova A I, Vagner V D, Grudyanov A I, Smirnova L E 2014 Clinical recommendations (protocols of treatment) Periodontal disease MГМSU 124 Moscow
[2] Herbert F W, Edith M R, Klaus H M 2008 Parodontologie Farbatlanten der Zahnmedizin p 548
[3] Shalak О V, Artyushenko N K 2012 Resective and reparative methods of periodontitis surgical treatment Chelovek Saint Petersburg 44
[4] Bulgakov A I, Galikeeva A S, Valeev I V, Khismatullina F R, Izgina E R, Khazieva L M, Mamedova G V, Saphiullina K S 2012 Examination of dental patient The main and additional methods training manual GBOU VPO BGMU Minzdravsotsrazvitiya Rossii Ufa 87

[5] Zahraa M N, Elhadi M A, Nada T K 2016 Microscopic differences in cementum structure and mineral composition of teeth extracted from patients with gingivitis, chronic periodontitis and aggressive periodontitis A preliminary comparative study International Journal of Dental Sciences and Research 4 (5) 90-4

[6] Hammarström L 1997 Enamel matrix, cementum development and regeneration Journal of Clinical Periodontology 24 (9) 658-68

[7] Timchenko P E, Timchenko E V, Volova L T, Frolov O O, Zybin M A and Bazhutova I V 2020 Raman spectroscopy of changes in the tissues of teeth with periodontitis Diagnostics 10(11) 876 (Preprint doi:10.3390/diagnostics10110876)

[8] Timchenko E V, Timchenko P E, Frolov O O, Yagofarova E F, Chernyy-Tkach K B, Zybin M A, Dolgushov G G 2019 Optical Methods for Periodontitis Early Rapid Diagnosis Electrical Engineering and Photonics (EExPolytech), IEEE 2019 298-300

[9] Timchenko P E, Timchenko E V, Volova L T, Frolov O O 2019 Spectral Analysis of Organic Components of Demineralized Bone Biografts Optics and Spectroscopy 126 (6) 769-75