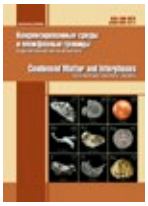

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Features of the molecular composition of dental biofilm in patients depending on the degree of caries and the method of its prevention: synchrotron FTIR spectroscopic studies

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Abstract

The article studies the molecular composition of dental biofilm in healthy people and patients with multiple caries lesions during several stages of exogenous and endogenous preventive measures.

The observed changes in the IR spectra registered during different stages of the experiment indicate a lack of balance between demineralisation and mineralisation of hard tissues resulting from different absorption mechanisms of agents applied exogenously and endogenously. All the observed changes result from the difference in the microbiota in healthy patients and patients with caries, as well as the difference in the microbiota caused by the impact of preventive agents on biofilm.

Keywords: Biofilm, Molecular properties, Endogenous and exogenous preventive methods, IR microspectroscopy, Synchrotron radiation

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1. Introduction

Current approaches to pathological processes including demineralisation of bones, caries, erosion, dental fractures and chipping, as well as to the prevention methods, stress the importance of studying the molecular composition and phase transformations on the enamel-dental biofilm interface at both micro and nano scales [1–4]. The biofilm serves as a buffer on the surface of dental enamel. All exchange processes between the organomineral matrix of enamel apatite [1, 5] and oral fluid containing active remineralisation agents (phosphate and calcium complexes) are performed through this buffer [4, 6]. Quantitative studies and control over oral pathologies, with biofilm serving as an analyte, require precise determination of the changes in its molecular composition.

Fourier-transform infrared spectroscopy (FTIR) is an effective and a highly precise method used for the analysis of biological systems [7, 8]. FTIR has already been successfully used in the analysis of biofilms (namely, for detection and identification of bacteria contained in biofilm) and showed promising results [7–10]. An undeniable advantage of FTIR over genetic analysis is the fact that the latter does not always provide information corresponding to the cells' phenotypes, while FTIR makes it possible to monitor molecular biochemical changes taking place in the analyte, including over time [7–10].

Earlier we demonstrated that synchrotron-radiation FTIR is practical for studying the secondary structure of proteins in biological fluids of the oral cavity and occurring pathological processes. The protein secondary structure determines their spatial conformation and therefore, under certain conditions, can be connected with pathological processes in the human body.

We should note that there is hardly any information about the changes in the molecular composition of human dental biofilm depending on the dental caries degree and the prevention methods used.

Therefore, the purpose of our study was to analyse the specifics of molecular composition of dental biofilm in healthy people and in patients with pathologies using synchrotron-radiation FTIR.

2. Materials and methods

2.1. Research design

In our study, we used dental biofilm samples obtained from patients under various cariogenic conditions. The first group included healthy people without dental caries. The second group included patients with multiple carious lesions in enamel (ICDAS 1–2).

During the first stage of the experiment, we collected biofilm samples from both groups after mechanical tooth cleaning.

During the second stage of the experiment, we collected biofilm samples from all the participants after they cleaned their teeth with a toothpaste containing dicalcium phosphate.

During the third stage of the experiment, patients took a mineral complex containing dicalcium phosphate for three days. Biofilm samples were taken after mechanical tooth cleaning.

2.2. Experimental unit

Molecular compositions of biofilm samples were studied using the equipment of the Australian Nuclear Science and Technology Organisation (Melbourne, Australia). The spectra were registered in the spectral range of 3100–900 cm^{-1} with a spectral resolution of 4 cm^{-1}. In order to do this, we used a Bruker Vertex 80v IR spectrometer and a Bruker Hyperion 2000 IR microscope equipped with a diamond high pressure attachment for quantitative micro-analysis.

3. Results and discussion

The FTIR absorption spectra registered for biofilm samples from healthy patients and from patients with various degrees of caries, including the spectra registered during different stages of the experiment, are demonstrated in Figs. 1 and 2. Analysis of the results demonstrated that all the spectra, regardless of the experimental group and the stage of the experiment, had the same set of maxima that can be attributed to characteristic molecular bonds. Preliminary analysis of spectral sets of certain samples also demonstrated that the IR spectra of the sets are practically identical. Therefore, in this article we give the average IR absorption spectra of biofilm samples.

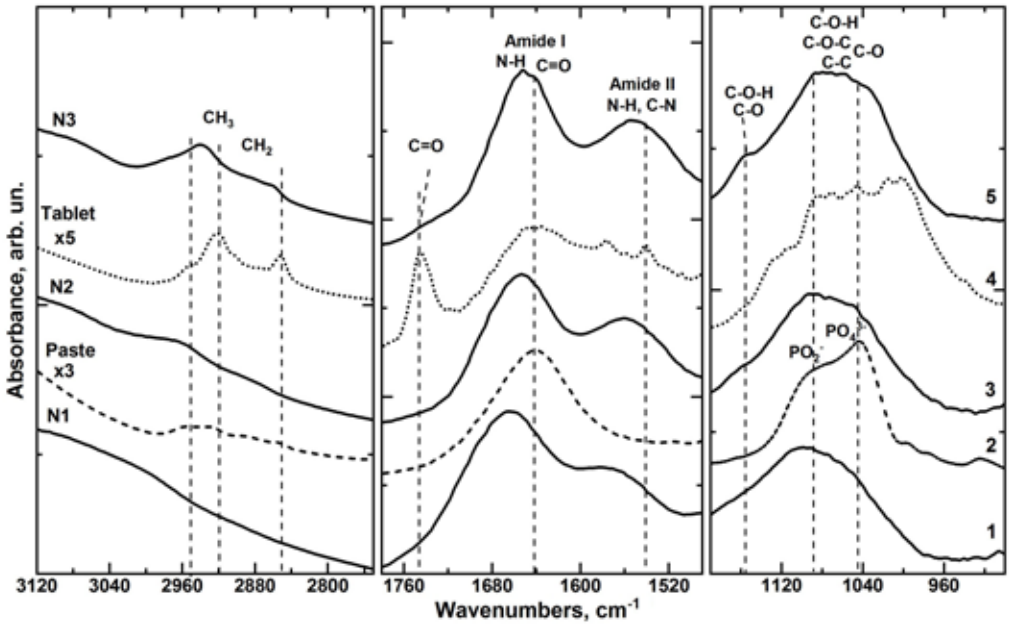

Fig. 1. IR absorption spectra of biofilm samples obtained from the enamel surface of healthy patients during different stages of the experiment: 1 – before applying preventive agents, 2 and 3 – after exogenous and endogenous preventive measures

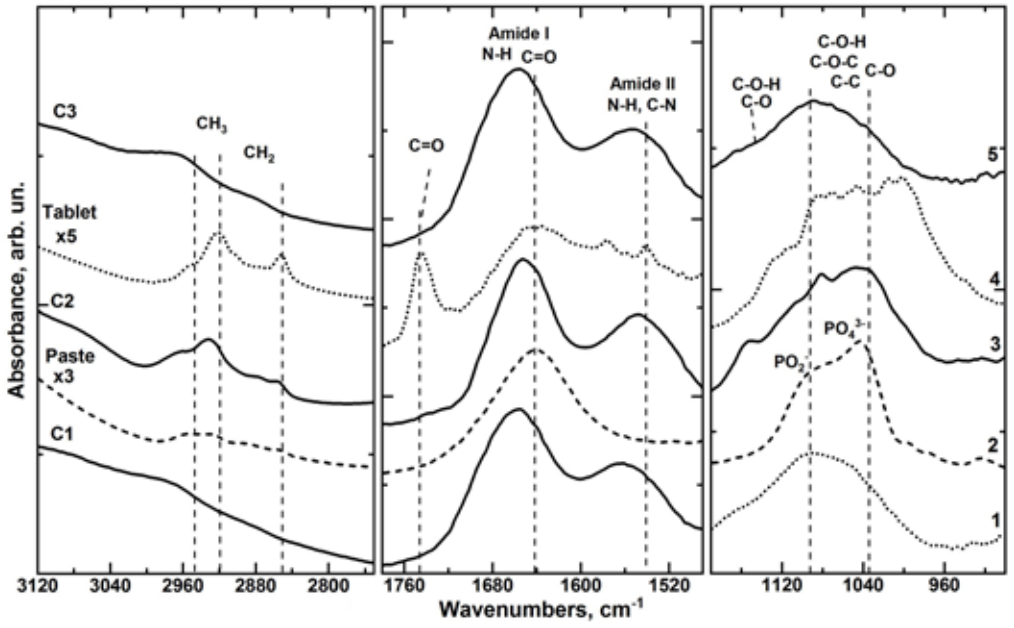

Fig. 2. IR absorption spectra of biofilm samples obtained from the enamel surface of patients with multiple caries lesions during different stages of the experiment: 1 – before applying preventive agents, 2 and 3 – after exogenous and endogenous preventive measures

The analysis of the experimental IR absorption spectra of biofilm samples was performed based on the existing data [7, 8, 11–15]. In these studies, vibrational IR spectroscopy was used to study the oral fluid, dental tissue, and biofilm in patients with various pathologies. The analysis demonstrated that the IR spectra of biofilm samples collected during different stages of the experiment have a set of characteristic vibrations, which can be attributed to molecular groups of various proteins, organic and inorganic agents, and oral microbiota.

The most significant changes in the relative intensities and profiles of the absorption bands observed in all the IR spectra of the biofilm samples were registered in the ranges 3120–2760, 1780–1500 cm^{-1}, and 1200–900 cm^{-1}. They are demonstrated in Fig. 1 and 2. When analysing the vibrational modes of the biofilm samples we noticed a group of bands localised in the range of 2950–2750 cm^{-1} and attributed to the vibrations of the C-H bonds of various fatty acids and lipids [7, 8]. We should note that the most significant changes in the relative intensity of these bands in the IR spectra of the biofilm samples were observed in the spectra registered for groups with different cariogenic situations (Fig. 2), when they used caries preventive agents. The analysis of the IR spectra of biofilm samples from healthy patients (fig. 1) demonstrated that the changes in the spectral region of 2950–2750 cm^{-1} are determined by the caries prevention method. This is easy to detect taking into account the characteristic spectral features of the preventive agents in the set range. At the same time, the IR spectra of biofilm samples from patients with multiple caries lesions (Fig. 2) demonstrated that significant changes in the molecular composition of biofilms occurred when putting preventive agents (toothpaste) into the oral cavity, while endogenous preventive methods did not have such an impact on the molecular composition of biofilms.

A similar tendency was observed when analysing the spectra in the range of 1200–900 cm^{-1}. A group of highly intense vibrations was observed associated with derivatives of phosphorus: phosphates, dicalcium phosphates, and phospholipids, which are important from the point of view of mineralisation processes [7, 8, 14]. We should note that the IR absorption spectra of biofilm samples demonstrated bands, whose occurrence and intensity in this range depend on the cariogenic situation and the stage of the experiment, i.e. the caries prevention method used. These modes include primarily the mode at 1082 cm^{-1} associated with PO_2^- by means of asymmetric and symmetric stretching vibrations of phosphate residues and phospholipids [7, 8, 14]. Another mode is located in the region of 1070–1020 cm^{-1}. It is presented as overlapping vibrational bands associated with organic derivatives of phosphates, dicalcium phosphate, and phosphatase by means of a C–O–P–O–C complex and cellular carbohydrate. Comparison of the results demonstrated that the use of a mineral complex in the form of pills or toothpaste leads to significant changes in the profile of the 1160–960 cm^{-1} band in biofilms obtained from healthy patients. In biofilms obtained from patients with multiple caries lesions, such changes were only observed immediately after applying the preventive agent whose composition results in significant changes in the molecular composition of biofilms in the given range. When a mineral complex in the form of pills was used, the molecular composition of biofilms did not change significantly after applying preventive agents (Fig. 3).

Another range of IR spectra that demonstrated significant changes was observed at 1780–1500 cm^{-1}. One of the spectral changes in the biofilm composition is the band at 1730 cm^{-1}, which can be attributed to bands (>C=O) of phospholipids, esters, and fatty acids, and corresponds to the characteristic region of proteins [7, 8, 11–13, 15]. At the same time, the most intense protein bands include the following: amide I vibrations (N–H, C=O) in the region of 1675–1615 cm^{-1}; amide II band (N–H and C–N) in the region of 1575–1520 cm^{-1}; according to [7, 8, 11–13, 15]. In the case of dental biofilms, these vibrational modes can also be attributed to peptides [7, 13].

We can see that the effect of preventive methods depending on the cariogenic situation is reflected by the position and shape of the amide I and amide II vibrational modes (Figs. 1 and 2). Thus, for the first (healthy) group, the use of a toothpaste and pills results in a significant (up to 14 cm^{-1}) shift of the amide I band towards the low-frequency region as compared to its position during the first stage of the experiment (without preventive agents). A similar tendency was observed for the group of vibration bands in the profile of the amide II. Here, for the healthy group, the use of a toothpaste and pills resulted in a significant (up to 25 cm^{-1}) shift of the band towards the low-frequency region as compared to its position during the first stage of the experiment (without preventive agents).

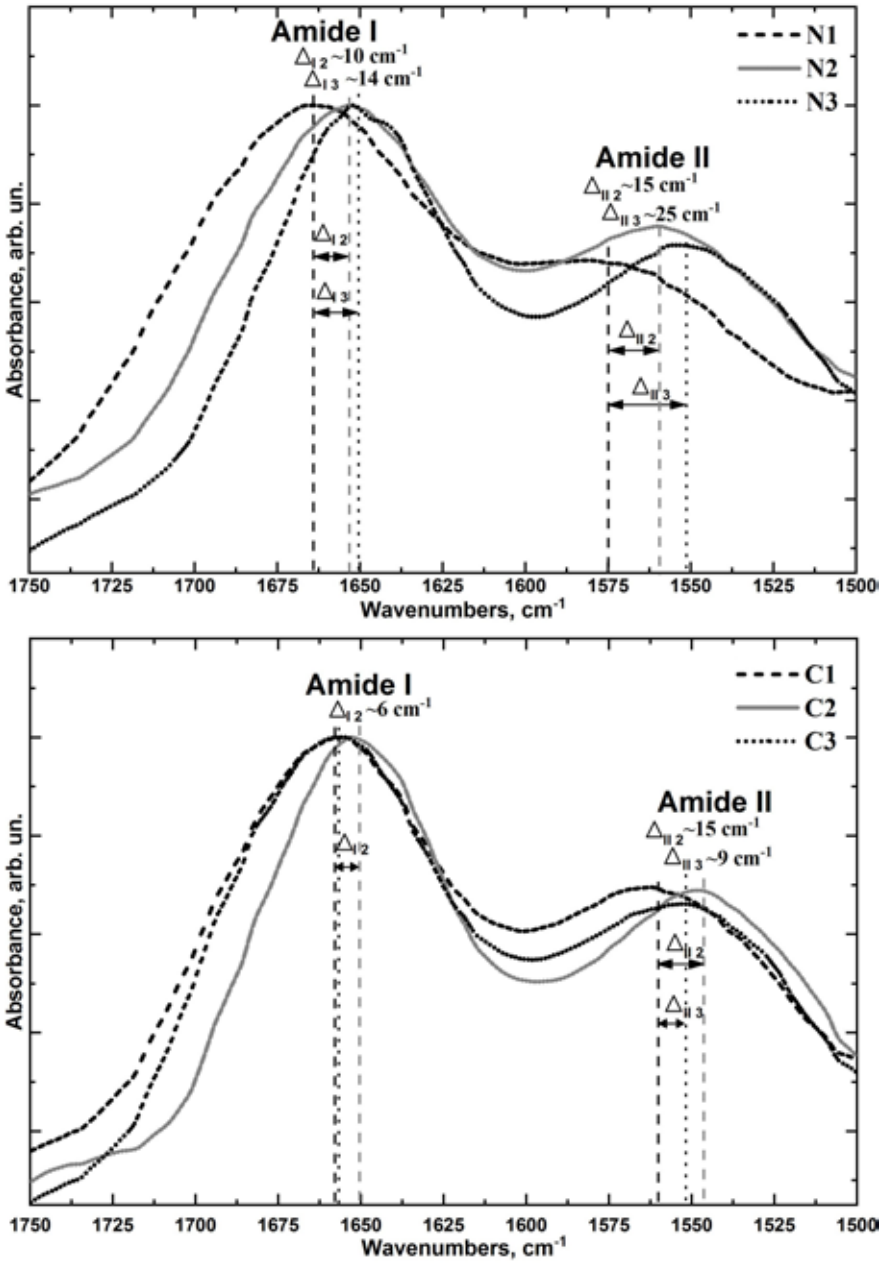

Fig. 3. Profiles of the amide I and amide II bands in the IR absorption spectra of the healthy (upper) and carious (down) group during different stages of the experiment: 1 – before applying preventive agents, 2 and 3 – after exogenous and endogenous preventive measures

For the group of patients with caries, these features were not that noticeable. Thus, a shift (up to 6 cm^{-1}) of the amide I band towards the low-frequency region was only registered during the second stage of the experiment (when toothpaste was used), while during the third stage (when pills were used) no shift was observed. At the same time, the shift of the maximum of the amide II band was 15 cm^{-1} during the second stage (when toothpaste was used), and only 9 cm^{-1} during the third stage (when pills were used).

The observed changes are caused by the changes in the biofilm molecular composition resulting from different cariogenic conditions and preventive methods used. Comparison of the results demonstrated that the changes in the profile of the amide II band (N–H and C–N) were greater than the changes in the profile of the amide I band. This corresponds to the

C–N vibrations, which can vary significantly depending on the factors affecting the molecular bonds.

The amide I band can be used to better monitor the changes in the protein secondary structure, because it is sensitive to such transformations. We should note that FTIR is often used to study protein conformation and aggregation processes *in vitro* [15, 16]. Based on the observed shifts in the frequency of the components of the secondary structure of the amide I band [11, 15, 17, 18] we can determine the impact of various factors on the protein conformation processes. Therefore, in our study we performed a precision comparative analysis of the IR spectroscopy data for the set frequency range of 1750–1500 cm^{-1}. A comparison of the spectra of biofilm samples obtained from both groups of patients demonstrated that the position and shape (half-width) of the high-frequency component of the amide I band in the region of 1700–1600 cm^{-1} depend on the cariogenic situation as well as on the preventive method. Thus, for the healthy patients, the use of a toothpaste and pills results in the shift of the amide I band towards the low-frequency region as compared to the first stage (without preventive agents), and to the reduction of the band's half-width from 55 cm^{-1} to 47 cm^{-1} and 37 cm^{-1} respectively. The shift and the reduction of the half-width were significantly greater, when pills were used. This is explained by the time the preventive agents spend in the oral cavity and the nature of their interaction with biofilm.

During the first stage of the experiment (without preventive agents), the IR spectra of patients with caries demonstrated that the position of the high-frequency component of the amide I band had already shifted by 7 cm^{-1} towards the low-frequency region as compared to that observed in the IR spectra of the healthy group. When toothpaste was used, there was a shift and a reduction in the half-width of the amide I band similar to that observed for the healthy group. However, when pills containing a mineral complex and dicalcium phosphate were used, there was no shift of the amide I band or any reduction in the half-width. More significant changes were observed for the amide II band. The spectral profile and the position of the band changed both when toothpaste and pills were used. We should note that when toothpaste was used, the shift of the amide II band was less significant, while the relative intensity of the maximum was lower than when pills were used. In the latter case the intensity was greater than the intensity of the spectral band of the samples obtained before using preventive agents.

The obtained spectral data indicated different conformation environment and secondary structure of biofilm proteins in patients with different cariogenic situations. The observed shift of the maximum of the high-frequency component of the amide I bands and the reduction of the half-width and redistribution of the intensity of components of the protein secondary structure was described in [11, 15, 19], namely as *random coil* (1648–1641 cm^{-1}) and α-helix (about 1660 $см^{-1}$). Changes in the molecular composition are also indicated by the changes in the relative intensity, frequency position, and spectral profile of the amide II band. All the observed changes result from the differences in the microbiota in healthy patients and patients with caries [20], when biofilm is affected by preventive agents [21].

The understanding of the changes in the molecular and phase compositions of dental tissues, oral fluid, and dental biofilm depending on the cariogenic situation and preventive methods makes it possible to take into account individual features of patients and perform effective treatment of caries, demineralisation, erosion, and dental attrition.

4. Conclusions

In our study, we used synchrotron-radiation FTIR to investigate the specifics of the molecular composition of dental biofilm after using exogenous and endogenous preventive methods in healthy patients and patients with multiple caries lesions.

The observed changes in the IR spectra indicate a lack of balance between demineralisation and mineralisation of hard tissues resulting from different absorption mechanisms of agents applied exogenously and endogenously. All the observed changes result from the difference in the microbiota in healthy patients and patients with caries, as well as the difference in the microbiota caused by the impact of preventive agents on biofilm.

Contribution of the authors

The authors contributed equally to this article.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. García-Godoy F., Hicks M. J. Maintaining the integrity of the enamel surface: The role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. *The Journal of the American Dental Association*. 2008;139: 25S–34S. https://doi.org/10.14219/jada.archive.2008.0352

2. Hicks J., Garcia-Godoy F., Flaitz C. Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 1). *Journal of Clinical Pediatric Dentistry*. 2004;28(1): 47–52. https://doi.org/10.17796/jcpd.28.1.yg6m443046k50u20

3. Hara A. T., Zero D. T. The caries environment: saliva, pellicle, diet, and hard tissue ultrastructure. *Dental Clinics of North America*. 2010;54(3): 455–467. https://doi.org/10.1016/j.cden.2010.03.008

4. Odanaka H., Obama T., Sawada N., Sugano M., Itabe H., Yamamoto M. Comparison of protein profiles of the pellicle, gingival crevicular fluid, and saliva: possible origin of pellicle proteins. *Biological Research*. 2020;53(1): 3. https://doi.org/10.1186/s40659-020-0271-2

5. Lee Y. H., Zimmerman J. N., Custodio W., Xiao Y., Basiri T., Hatibovic-Kofman S., Siqueira W. L. Proteomic evaluation of acquired enamel pellicle during in vivo formation. *PLOS ONE*. *Public Library of Science*. 2013;8(7): e67919. https://doi.org/10.1371/journal.pone.0067919

6. Meyer F., Enax J., Epple M., Amaechi B.T., Simader B. Cariogenic biofilms: development, properties, and biomimetic preventive agents. *Dentistry Journal*. 2021;9(8): 88. https://doi.org/10.3390/dj9080088

7. Chirman D., Pleshko N. Characterization of bacterial biofilm infections with Fourier transform infrared spectroscopy: a review. *Applied Spectroscopy Reviews*. 2021;56(8–10): 673–701. https://doi.org/10.1080/05704928.2020.1864392

8. Gieroba B., Krysa M., Wojtowicz K., Wiater A., Pleszczyńska M., Tomczyk M., Sroka-Bartnicka A. The FT-IR and Raman spectroscopies as tools for biofilm characterization created by cariogenic streptococci. *International Journal of Molecular Sciences*. 2020;21(11): 3811. https://doi.org/10.3390/ijms21113811

9. Azam M. T., Khan A. S., Muzzafar D., Faryal R., Siddiqi S. A., Ahmad R., Chauhdry A. A., Rehman I. U. Structural, surface, in vitro bacterial adhesion and biofilm formation analysis of three dental restorative composites. *Materials*. 2015;8(6): 3221–3237. https://doi.org/10.3390/ma8063221

10. Cheeseman S., Shaw Z. L., Vongsvivut J., Crawford R. J., ... Truong V. K. Analysis of pathogenic bacterial and yeast biofilms using the combination of synchrotron ATR-FTIR microspectroscopy and chemometric approaches. *Molecules*. 2021;26(13): 3890. https://doi.org/10.3390/molecules26133890

11. Baldassarre M., Li C., Eremina N., ... Barth A. Simultaneous fitting of absorption spectra and their second derivatives for an improved analysis of protein infrared spectra. *Molecules*. 2015;20(7): 12599–12622. https://doi.org/10.3390/molecules200712599

12. Barth A., Haris P. I. *Biological and biomedical infrared spectroscopy*. IOS Press; 2009. 449 p.

13. Matthäus C., Bird B., Miljković M., Chernenko T., Romeo M., Diem M. Infrared and Raman microscopy in cell biology. *Methods in Cell Biology*. 2008: 275–308. https://doi.org/10.1016/S0091-679X(08)00610-9

14. Ren Z., Do L. D., Bechkoff G., ... Buchet R. Direct determination of phosphatase activity from physiological substrates in cells. *PLoS ONE*. 2015;10(3): e0120087. https://doi.org/10.1371/journal.pone.0120087

15. Yang S., Zhang Q., Yang H., Shi H., Dong A., Wang L., Yu S. Progress in infrared spectroscopy as an efficient tool for predicting protein secondary structure. *International Journal of Biological Macromolecules*. 2022: 175–187. https://doi.org/10.1016/j.ijbiomac.2022.02.104

16. Ripanti F., Luchetti N., Nucara A., Minicozzi V., Venere A. D., Filabozzi A., Carbonaro M. Normal mode calculation and infrared spectroscopy of proteins in water solution: Relationship between amide I transition dipole strength and secondary structure. *International Journal of Biological Macromolecules*. 2021;185: 369–376. https://doi.org/10.1016/j.ijbiomac.2021.06.092

17. Seredin P., Goloshchapov D., Ippolitov Y., Jitraporn Vongsvivut. Spectroscopic signature of the pathological processes of carious dentine based on FTIR investigations of the oral biological fluids. *Biomedical Optics Express*. 2019;10(8): 4050–4058. https://doi.org/10.1364/BOE.10.004050

18. Miller L. M., Bourassa M. W., Smith R. J. FTIR spectroscopic imaging of protein aggregation in living cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2013;1828(10): 2339–2346. https://doi.org/10.1016/j.bbamem.2013.01.014

19. Seredin P., Goloshchapov D., Ippolitov Y., Vongsvivut J. Comparative analysis of dentine and gingival fluid molecular composition and protein

conformations during development of dentine caries: A pilot study. *Vibrational Spectroscopy*. 2020;108: 103058. https://doi.org/10.1016/j.vibspec.2020.103058

20. Kriebel K., Hieke C., Müller-Hilke B., Nakata M., Kreikemeyer B. Oral biofilms from symbiotic to pathogenic interactions and associated disease –connection of periodontitis and rheumatic arthritis by peptidylarginine deiminase. *Frontiers in Microbiology*. 2018;9. https://doi.org/10.3389/fmicb.2018.00053

21. Meyer F., Amaechi B. T., Fabritius H.-O., Enax J. Overview of calcium phosphates used in biomimetic oral care. *The Open Dentistry Journal*. 2018;12(1): 406–423. https://doi.org/10.2174/1874210601812010406

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