Spectroscopic examination of dentine and gingival fluids and their diagnostic capability for the preventive treatment of pathology carious processes in dentine

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Abstract. IR-spectra of the samples of the dentine and gingival fluids obtained with the use of synchrotron radiation can be applied for the diagnostics of the pathological processes of the caries character in a dentine. Vibrations within the range of 2100-2050 cm\(^{-1}\) observed in the spectra of dentine and gingival fluids characteristic of thiocyanates mean the development of the carious pathology. At the same time vibration modes of the carboxyl group of a complex ether detected within the range of 1765 – 1725 cm\(^{-1}\) in the spectra of dentine and gingival fluids certainly confirm the development of caries in dentine. These spectrometric signatures can be certainly and reliably detected also in the gingival fluid, indicating at the fact that the changes proceeding in its molecular composition are referred just to the development of pathological processes in the deep dental tissues.

1. Introduction

In spite of the fact that dentine caries is quite often a consequence of the following development of pathology process starting in the dental enamel, successful detection and monitoring of the changes in deep dentine tissues is rather a complicated problem [1,2]. Effective pathology screening of the sort was shown to rely on analysis of biological mouth cavity fluids [2–4]. However, microvolumes of a sample are necessary for this kind of procedure, which along with minor changes in the molecular composition of biological fluids requires the highly sensitive method of detection of inflammation markers at the stage of pathology development [2]. The most suitable technique is Fourier Transform Infrared spectroscopy (FTIR) that is non-invasive, rapid, precise and highly selective, allowing the investigation of different type of pathology through human saliva and gingival fluid analysis [2,5,6]. Meanwhile, an ideal candidate for the role of the screening object in case of dentine caries could be dentine fluid [7]. Dentine fluid is a derivative of blood plasma, containing serum proteins, immunoglobulins and dissolved mineral substances [8]. Dentine fluid moves from the tooth pulp, fills the branched proliferating dentine channels and actively interacts with dentine tissue. Moreover, dentine fluid is quite often serves as a nutrition for microorganisms thus resulting in another mechanism of the caries development different from that in enamel [1]. In this case bacterial metabolites diffuse through the dental tubules [7], and it is highly probable that markers of pathological processes can enter the gingival sulcus through the dentine tubules and thus mix with fluid from the sulcus, which is serum transudate [7]. The main complexity of a diagnostic approach of
dentine fluid is a difficult algorithm of its extraction. Extraction of the fluid from the gingival cervicular groove used for the diagnostics of dentine pathology is much more simple task and its molecular analysis with the following selection of markers indicating at the development of pathological processes can be performed with the use of molecular identification technique [9]. By comparing the changes in the molecular composition of dentine and gingival fluids obtained by FTIR, it is possible to define markers in gingival fluid that are referring to caries processes in dentin.

Therefore, the aim of our work is a search for a spectroscopic signature concerned with the pathology processes of the carious type in dentine basing on FTIR investigations of dentine and gingival fluids.

2. Materials and methods
10 humans participated in the study. On examination, each participant had teeth with lesion foci related to primary and secondary caries at the stage corresponding to code 1 and 2 according to the International Caries Detection and Assessment System (ICDAS). Participants with caries detected after cofferdam separation of a tooth underwent preparation of both the enamel and dentine using a micro-engine air tip of a spherical doped tungsten–vanadium steel dental drill rotating at 4000 revolutions per minute. After creating a fissure in the masticatory surface in the tooth up to the dentine opening, the infected de-mineralized layer of yellowish dentine was observed. Subsequently, if the examination confirmed the development of dentine caries, dentine fluid was sampled from the prepared cavity using a microcapillary tip.

The capillary effect was used for obtaining microvolumes of fluid as in [10]. The applied tip represents a microcapillary was filled with homogenized potassium bromide (KBr) powder. The KBr was used as an inert carrier of the investigated fluid, while its choice as a filler was based on the absence of absorption bands within a wide IR spectrum range. For each of the patients a sample of dentine fluid and a sample of a fluid from the gingival groove were taken by the microcapillary that was attached to a sterilized syringe. The study of molecular composition of the samples was performed with the use of Infrared Microspectroscopy (IRM) beamline (Australian synchrotron, Victoria, Australia), using a Bruker Vertex 80v spectrometer coupled with a Hyperion 3000 FTIR microscope. All the synchrotron FTIR spectra were recorded within a spectral range of 3800‒700 cm⁻¹ using 4 cm⁻¹ spectral resolution. The most intensive IR-bands are in the region of 2250-850 cm⁻¹ (figure 1).

![Figure 1. Comparison of IR spectra within the range of 2200-850 cm⁻¹ averaged over the groups of the samples of the dentine (curve 1) and gingival (curve 2) groups of fluids taken from a human.](image-url)
Spectral data processing, and all manipulations of the spectra (removal of the background and correction for atmosphere conditions), averaging of the spectra and data integration, and all calculations, were performed using OPUS 7.2 software (Bruker Optik GmbH, Ettlingen, Germany).

3. Results
The experimental IR-spectra demonstrated that the spectra of the participants’ same-type samples comprised absolutely one and the same set of vibration modes (figure 1). Furthermore, these spectra differed from each other non-significantly only by changes in the vibration band intensity. IR-spectra of the samples of dentine fluid and fluid from gingival groove averaged over the groups of participants taking place in the experiment are presented in figure 1. Confidential interval of the averaged absorbance spectra for each group of participants was no more than 2%. Analysis of the obtained data and interpretation of IR spectra were performed basing on the results of a number of the reference works where the samples of the biological fluids taken from the oral cavity were examined by FTIR as well as proteins and amino acids [6,11,12].

It should be noted that the initial stage of the caries development that is not clinically diagnosed in practice (stage 1 or 2 in ICDAS scale) can be detected by IR-spectroscopy technique due to the presence of a number of characteristic vibration bands in the spectrum of the oral cavity fluid. Vibration band in the range of 2100-2050 cm⁻¹, that is present in the spectra of as dentine as gingival fluids can be attributed to thiocyanates [3,4], which prove to be indicators of pathology processes in the oral cavity and their content increases in case of caries and paradont diseases [3]. As for the IR-band in the range of 1765 – 1725 cm⁻¹, then according to the data of [13] this vibration mode corresponds to >C=O vibration and is attributed to the carboxylic group of a complex ether (ether carboxyl). In [14] it was shown that ethers are more often observed in the carious than in the intact sound hard dental tissue. The next vibration band observed at 1171 - 1160 cm⁻¹, is attributed to carbohydrates and an increase of their level in the oral fluid also indicates at the development of carious process.

However, despite an evident presence of these vibration bands in the IR spectrum which are attributed to the occurrence of pathological processes in the oral cavity, their low intensity does not allow identifying pathology with a sufficient accuracy. Therefore certain spectral features that are manifested in the profiles of the most intensive vibration bands of the following groups require more detailed analysis [2,3]. The most intensive group of vibrations arranged within the interval of 1725 - 1190 cm⁻¹, is attributed to proteins. Within this group some additional bands of secondary amides can be separated: Amide I (C=O stretch vibrations within the range of 1725 – 1590 cm⁻¹), Amide II (N-H bending and C–N stretch vibrations within the range of 1590 – 1500 cm⁻¹) and Amide III (C–N stretch, N–H bending modes in the range of 1350-1190 cm⁻¹), as well as the vibrations of CH₂/CH₃ groups arranged at 1480 – 1350 cm⁻¹ [4,6,11]. The following large group of vibration bands localized within the limits of 1130 – 900 cm⁻¹, is attributed to the molecular bonds related with phosphates, glycerophosphartes and phospholipids [5], as well as with carbohydrates and nucleic acid phosphate [6]. It should be noted that for the samples of dentine and gingival fluids this group of bands involves a wide set of vibrations related with the mineral component (phosphate derivatives).

4. Discussion
The analysis of the experimental IR-data reveal that a special attention in the IR-spectra of the biological fluids within the oral cavity should be focused on the transmission bands arranged at 2100-1050 cm⁻¹, 1765 – 1725 cm⁻¹, 1171 – 1160 cm⁻¹ because of its connection with carious pathology [3,4]. Basing on the approach checked in a number of various works [5,15,16], there is a possibility to compare molecular composition of the dentine and gingival fluids taken from the patients with pathology of a deep dentine tissue of cariogenic character. Using the proposed approach the following coefficients (R1-R3) were calculated separately for biological fluids from the mathematical estimation of the area under the curve of IR-spectra bands. It is known that developing of pathology process can
reflect to the Amide structure and have an influence on its form. Thus, first coefficient $R_1$ (Amide II/Amide I) was calculated from the ratio of the integral intensity for the band Amide II (CN stretching, NH bending vibrations) in the range of 1600 - 1458 cm$^{-1}$ to the integral intensity of Amide I band (C=O stretching) in the range of 1720-1600 cm$^{-1}$. Second coefficient $R_2$ – thiocyanate/protein, proposed in [16], was calculated as the ratio of integral intensity between the vibration band of $\tilde{N}=\tilde{C}=\tilde{S}$, arranged in the interval of 2100 – 2050 cm$^{-1}$, attributed with thiocyanate, to the integral intensity of the amide bands (Amide I and Amide II) in the range of 1720-1485 cm$^{-1}$. The ratio of Ether/Amide I ($R_3$ coefficient) was calculated from the ratio of the integral intensity between the carboxylic group of a complex ether (ether carboxyl) within the range of 1765 – 1725 cm$^{-1}$ and the integral intensity of Amide I band (in the range of 1720-1600 cm$^{-1}$). Results of the calculations for $R_1$ – $R_3$ are presented in figure 2.

Analyzing the obtained results one should note that while developing of caries in dentine the ratio of CN and NH bonds relative to C=O bonds ($R_1$ coefficient) in the gingival and dentine fluids does not actually change, thus indicating at the correlation of $R_1$ coefficient with the protein component.

![Figure 2](image_url)

**Figure 2.** Calculated $R_1$-$R_3$ ratios obtained for the gingival and dentin fluid: (a) - Amide I/Amide II ratio ($R_1$); (b) - Thiocyanate/ Amides I+II ratio ($R_2$); (c) - Ether/Amide I ratio ($R_3$).

Obvious signature changes in the molecular composition of biological fluids connected with the development of caries in dentine, can be detected by the analyzing $R_2$ and $R_3$ coefficients. It can be easily seen that an increase in thiocyanates level ($R_2$) as well as that one of complex ethers ($R_3$) that accompanies caries development [3,4], is observed in the samples of both dentine and gingival fluids (figure 1). It is clear that these coefficients are of a greater value for the dentine fluid. Note, that these spectrometric signatures can be certainly and reliably detected also in the gingival fluid, indicating at the fact that the changes proceeding in its molecular composition are referred just to the development of pathological processes in the deep dental tissues. Low-intensive characteristic vibrations of thiocyanates, esters and carbohydrates detected in the work with the use of synchrotron radiation in the IR-spectra of the biological fluids associated with the appearance of a carious pathology in the dentine tissues along with the calculated coefficients $R_1$–$R_3$ will be available for the elaboration of algorithms applied in the study of carious pathology with the use of the routine laboratory IR-spectrometers.

5. Conclusion

IR-spectra of the samples of the dentine and gingival fluids obtained with the use of synchrotron radiation can be applied for the diagnostics of the pathological processes of the carious character in a dentine. Vibrations within the range of 2100-2050 cm$^{-1}$ observed in the spectra of dentine and gingival fluids characteristic of thiocyanates mean the development of the carious pathology. At the same time vibration modes of the carboxyl group of a complex ether detected within the range of 1765 – 1725 cm$^{-1}$ in the spectra of dentine and gingival fluids certainly confirm the development of caries in dentine. One should note that the signatures of the development of carious pathology in dentine found
in the spectra of the dentine fluid can be easily detected without its labor-exhaustive and hardly expedient extraction since they are also present in the gingival fluid, and its sampling for the screening is not such a difficult problem. Therefore based on the suggested method and identified caries markers screening method of deep dentine caries can be developed.

Acknowledgments
This work was supported by the grant of Russian Science Foundation, grant number 16-15-00003.

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