The study of molecular composition in biomimetic interface of biocomposite/dentin

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Abstract. The aim of the study is the problem of formation of the biomimetic interface between the dental product and dentin of the human tooth as well as the investigations of molecular-chemical features in biointerface with the use of molecular multivariate IR-visualization. The data on synchrotron IR-mapping made enabled to differentiate the regions of sound dentin tissue and biomimetic transition layer and also to determine molecular groups responsible for the process of integration.

1. Introduction
Biomimetic remodeling of enamel and dentin of teeth demonstrates that in order to improve integration of dental products with the hard dental tissue it is required to form nano-filled biomimetic buffer layers. Furthermore, the composition of buffer layers should involve nanocrystalline hydroxyapatite (n-HAP) and amino acid matrix[1]. Incorporation of nano-HAP with various defect structure into the composition of a hybrid layer, as well as a number of polar amino acid help to resemble characteristic for the enamel matrix [1–4]. Such strategy makes it possible to improve integration of synthetic materials and natural tissue [5–7], while the transition layer ceases to be a weak link of the restoration dentistry [8]. Therefore, investigations of formation of the biomimetic biocomposite/dentin interface and determination of its molecular composition with the use of FTIR-microspectroscopic visualization technique was the aim of our study.

2. Materials and methods
The study was performed with tooth samples removed from patients in the age of 20-40 according to the orthodontic criteria. With the use of Er,YAG laser the hard dental tissue was prepared up to dentin. Next, dentin conditioner, bio-primer, universal adhesive and compomer were deposited layer by layer on the prepared surface of dentin[6]. Treatment processes were performed in the conditions of alkaline environment with pH > 11 for the formation of the buffer hybrid layer. To promote formation of the bond with the apatite of dentin polar amino acids – L-lysine hydrochloride and L-arginine hydrochloride were additionally introduced in the composition of bio-primer. Nanocrystalline
carbonate-substituted hydroxyapatite (n-CHAP) was introduced as a filler in the universal adhesive [9].

In accordance with the requirements of the microspectroscopic research techniques for the geometry of the samples, we prepared plane-parallel segments of the teeth. Molecular composition of the interface samples was studied with the use of synchrotron ATR FTIR-microspectroscopy[10]. The synchrotron FTIR experiment was performed at Infrared Microspectroscopy (IRM) beamline (Australian synchrotron, Victoria, Australia), using a Bruker Vertex 80v spectrometer coupled with a Hyperion 3000 FTIR microscope and a liquid nitrogen-cooled narrow-band mercury cadmium telluride (MCT) detector (Bruker Optik GmbH, Ettlingen, Germany). The synchrotron FTIR spectra were recorded within a spectral range of 3800–700 cm\(^{-1}\) using 4-cm\(^{-1}\) spectral resolution. Blackman-Harris 3-Term apodization, Mertz phase correction, and zero-filling factor of 2 were set as default acquisition parameters using OPUS 8.0 software suite (Bruker Optik GmbH, Ettlingen, Germany). A subsequent synchrotron macro ATR-FTIR mapping measurements were performed on specific areas of interest found in the prior overview map where a good contact with the Ge ATR crystal was achieved, using a smaller step interval of 1 μm and 32 co-added scans.

3. Results

The proposed technique of molecular analysis mapping of biointerface was applied for the region between biomimetic apatite layer and dentin tissue of the human tooth (60×30 μm) (Figure 1,2) with a spatial resolution of 1 μm. Basing on the color coding one-dimensional IR-maps were designed that demonstrate the distribution of molecular group of ether –COOCH\(_3\) (1762-1701 cm\(^{-1}\)) associated with compomer (Figure 1), vibrations of molecular groups CH\(_2\), CH\(_3\), the bands of Amid III and collagen which are present in dentin (Figure 2), the distribution of molecular group Amid I (1685-1611 cm\(^{-1}\)) (Figure 3) and mineral (phosphate) PO\(_4\) component (1113-961 cm\(^{-1}\)) (Figure 4).

The obtained FTIR maps (see Figure 1,2) provide information on the spatial distribution of the etheric (bioprimer-adhesive-compomer) and protein (collagen) components in the region of dentin/compomer. In the area of dentin one can easily observe the bands with the average color intensity (Figure 1). Their appearance in the map is caused by the presence of dentin tubules. Analysis of FTIR map of the distribution for Amid I band in the range of 1685-1611 cm\(^{-1}\) (Figure 3) does also support this statement. It means penetration of bioprimer components and universal adhesive into dentin. This coincides with the results of works concerned with the microscopic investigations of dentin/adhesive interface [11]. The detailed analysis of the data on FTIR-micromapping of the interface areas revealed the presence of a hybrid layer between the area of a sound dentin and the area of adhesive/dental material. It should be noted that the hybrid layer is composed both of the
components of bioprimer and adhesive as of the collagen fibers and enamel apatite, that follows from the analysis of spatial distribution of the vibration bands intensities: 1762-1701 cm\(^{-1}\), 1485-1376 cm\(^{-1}\) and 1113-961 cm\(^{-1}\), respectively (Figure 1,2,4).

Joint analysis of the spectral data array (position and intensity of Amid I and phosphate bands) in the area of hybrid layer (Figure 2,4) indicates at the appearance of the interphase interaction [12]. Considering the characteristics of preparation of the dentin tissue with the erbium laser this interaction is realized as a result of formation of the demineralized and disorganized area in dentin and the following penetration of bioprimer and adhesive components into dentin. The following hierarchical cluster analysis of the spectral data [13] demonstrated that the interaction between dental product and the natural hard tissue of dentin is realized by the biomimetic buffer layer that is bound with partially demineralized dentin matrix (Figure 2,3), and organic-mineral interaction occurs within the 10 \(\mu\)m region. Analysis of FTIR map of the mineral (phosphate) component (Figure 4) in the enamel apatite demonstrated correctness of this assumption. Decrease of intensity in the area of dentin means demineralization of the hard dental tissue in the direction of dentin tubules. At the same time non-zero intensity of the phosphate component within the area of hybrid layer (Figure 4) is explained by the presence of nanocrystalline hydroxyapatite in the adhesive. It is known [14], that inclusion of various remineralization agents in the adhesive systems is just a factor which already allows formation of the hybrid interface based on polymer, collagen and resin substituting native tissue of dentin at the boundary between dentin and adhesive [1]. Such approach provides positive results and it enables to form stable bounds between adhesive and dentin.

3. Conclusion
Applying synchrotron IR-mapping it was shown that our elaborated biomimetic buffer layer involving n-CHAP and the complex of the main polar amino acids which are characteristic for the natural dental tissue can produce the functional bond as with the dental compomer as with natural dentin. The use of biomimetic buffer layers makes it possible to achieve an excellent biocompatibility during repair of dental structure and it can form the basis for the new therapeutic approach in dentistry.

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