A Synchrotron Study of Molecular and Chemical Interaction at the Dental Material/Biomimetic Composite/Native Hard Dental Tissue Interface

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Abstract. Based on the technology of molecular multidimensional IR-synchrotron visualization, the paper explores integration of a new generation of biomimetic composites that regenerates a mineral organic dental enamel complex with native human hard dental tissues and dental cement. The data of spectral molecular visualization was obtained from the area of the healthy hard tissue (enamel/dentine) – biomimetic transition layer – dental material/adhesive interface. The resulting data is indicative of chemical differentiation of functional groups of all the materials at the biomimetic system/natural hard tissue and prove the chosen method to be effective in analyzing integration of dental concrete and new generation biomimetic composites.

1. Introduction
Despite high levels of adhesion and strength, modern composites and bonds employed in restorative dentistry are known to have a low affinity with native human dental tissue with regards to chemical composition and morphological organization [1].

The most viable method for designing a synthetic material – enamel/dentin interface of a high-quality is to come up with a biomimetic material mimicking the composition, micro and nanostructure of hard dental tissues as part of the biomimetic approach [2,3]. Introduction of calcium hydroxyapatite (HAP) into a biocomposite is well-known to improve integration of synthetic materials [4]. Additionally, inclusion of polar amino acids of an enamel matrix [5,6] into biocomposites enables mimicking of a certain enamel or dental dentin area by means of a biomimetic material as well as improves their adhesive and strength characteristics [7].

Evaluation of bond integration with dental tissues as well as analysis of an emerging adhesive/enamel interface can be performed by means of the Fourier-transform infrared spectroscopy (FTIR) which allows a molecular composition and thin structural properties of biological objects to be identified based on analyzing molecular oscillation bands in IR-spectra that are specific to particular chemical groups [8]. While studying biological objects, large datasets can be obtained from a microarea of a sample due to a microscope included into a measurement scheme as well as a source of synchrotron radiation [9]. Using a spectrum massive obtained, IR-microspectroscopic map of an object providing a wealth of information on molecular bonds in a sample and their distribution in space can be designed.
Therefore the objective of the paper is to investigate molecular and chemical features of a dental material – biomimetic buffer layer – hard dental human tissue interface by means of multidimensional visualization of IR-microspectroscopy data.

2. Materials and methods

2.1. Samples preparation

In order to obtain biomimetic modelling materials, we employed a system containing the following: hyaluronic acid (0.01-0.05 wt. %), L-histidine (0.01-0.2 wt. %), L-lysine hydrochloride (0.05-0.4 wt. %), L-arginine hydrochloride (0.2-1.6 wt. %) as well as ethylene glycol methyl ether (30-85 wt. %), diglycidyl dimethacrylate (1-15 wt. %), urethane dimethacrylate (1-15 wt. %), ethyl alcohol (2-20 wt. %) and water (the remainder). In order to design an environment which is high-affinity to native dental tissues, a synthetic carbonate substitute calcium hydroxyapatite (CHA) was added in the ratio of 1 ml of the mix - 0.01 g CHAP to meet a range of the characteristics of the enamel apatite and human dental dentin [5]. In order to fix the above buffer system, a universal adhesive was used for a bioactive bonding system. The CHAP resulting from the current study was added in the ratio 1 ml of the adhesive – 0.01 g CHAP into the universal adhesive for improving the affinity. The HAP components were mixed with those of the buffer system and the adhesive by means of the ultrasonic homogenizer QSonica for 30 seconds.

The integration of the buffer layers based on the biocomposites was examined using samples of teeth removed from patients aged 18 to 45 in accordance with orthodontic treatment plan. The teeth were prepared and the biomimetic buffer system was applied according to the scheme which follows. In the first stage the enamel and dentin were prepared and the filling void was formed. In the second stage the enamel was etched for 60 seconds using an enamel etching gel based on a 37% phosphorous acid followed by water rinsing and air flow drying. The dentin was then processed with the dentin conditioner based on a hyaluronic acid mix (0.01-0.05 wt. %), L-histidine (0.01-0.2 wt. %), L-lysine hydrochloride (0.05-0.4 wt. %), L-arginine hydrochloride (0.2-1.6 wt. %) for 20-30 seconds. After that, the void surface was dried. In the third stage the biocomposite buffer system resulting from the current study was evenly distributed into the walls of the obtained void and 20 seconds later it was dried using an air compressor. A universal light-curing adhesive containing CHAP which was then preliminarily photopolymerized for 20 seconds was applied onto the buffer layer surface prepared in such a fashion. Finally, in the fourth stage a minute later a commercial compomer restorative material containing the adhesive was applied onto the biomimetic buffer layer. Taking into consideration the research methodology (IR-microspectroscopy) requirements for sample geometry, parallel slices of the segments of the restored teeth samples were prepared.

2.2. Method

The molecular composition of the samples in the dental material – biomimetic buffer layer – native hard human dental tissue interface was examined using synchrotron IR-microspectroscopy as well as attenuated total reflection (ATR). The study was conducted on the Infrared Microspectroscopy (IRM) (the Australian synchrotron) containing a Hyperion 3000 IR-microscope (Bruker) and an ATR element with a germanium prism [9]. In Fig. 1a a rectangular area indicates an image of the investigated interface area used to obtain the absorption IR-spectra in the range from 3800-700 cm\(^{-1}\) (Fig. 1b).

Using the possibilities offered by the IR-microscope and the OPUS software, following molecular mapping one-dimensional IR-images (IR-maps) were designed in the sample area sized 100x100 μm (Fig. 1a) with a step 2μm based on colour coding of the intensities of the absorption bands of the IR-spectra. The maps show the distribution of the intensities of the molecular group and thus its concentration at a certain point of the investigated sample.
Figure 1. (a) - 100x100 mkm section containing the interphase border of a light-cured dental material / biomimetic composite / enamel from which an array of spectra was obtained. (b) - typical infrared absorption spectrum from the interface region.

3. Results

Through the course of the study of the interphase interface area (Fig. 1a) a set of the main oscillation modes was identified in the IR-spectrum (Fig. 1b) between the light-curing dental material, biomimetic composite as well as enamel and dental dentin.

Fig. 2a presents the IR distribution map of the PO₄ molecular group in the interface area (Fig. 1b). The data analysis shows that the dental material area does not contain any phosphate groups. The entire area interfacing with the enamel where a non-zero intensity of the active oscillations in the range 1163-981 cm⁻¹ (PO₄ band) is sized ~30 μm and is indicated in Fig. 2a with a dotted line.

The IR-image presented in Fig. 2b was generated for obtaining extra information on the interphase interface area. This IR-map provides the data on the distribution of the intensities of the CN, NH, C=O, CH₂/CH₃ molecular groups in the range 1718-1358 cm⁻¹ (Fig. 1b) which are part of the collagen as well as the Amide I and Amide II components that are contained in the organic component of the enamel/dentin and biomimetic buffer layer. Analytical processing of the IR-map (Fig. 2a) enables us to conclude that the distribution of the organic component in the transition buffer layer is more homogeneous compared to that of the phosphate groups. The IR-spectrum in Fig. 1b contains the absorption band in the area 1725 cm⁻¹. This oscillation is characteristic of IR-spectra of dental materials.
and belongs to the ether molecular group (–COOCH₃) [10]. Additionally, this absorption band is not overlapped by the other oscillations, which allows us to design the IR-image (Fig. 3a).

![Image](https://example.com/figure3.png)

Figure. 3 The characteristic IR image obtained on the basis of color coding of the intensity of the absorption band: (a) of compomer material 1725 cm⁻¹ and (b) of Amide III 1269-1224 cm⁻¹

Fig. 3a suggests that the maximum distribution of the intensities of the ether group (–COOCH₃) coincides with the position of the dental material observed in the optical shot (Fig. 1a).

The analysis of the resulting experimental spectrum from Fig. 1b allows an intensive absorption band Amide III to be identified in the area 1269-1224 cm⁻¹ and the IR-map which only belongs to the transition buffer layer (Fig. 3b) to be designed. It should be noted that the above oscillation (Fig. 1b) is not overlapped by the absorption of the other functional groups and can act as a reference of the biomimetic composite. The comparison of the data of the optical image (Fig. 1a) and IR-map in Fig. 3b is clearly indicative of the fact that the molecular group 1269-1224 cm⁻¹ is mainly distributed only in the narrow area of the sample containing the light-curing material – biomimetic buffer layer – enamel/human dental dentin interface.

4. Discussion

While analyzing IR-images of the area of the heterophase interface, it has to be remembered that the area in the IR-map of the phosphate group (Fig. 2a) with the intensity ranging from 1.0 to 7.0. is of certain interest. This area belongs to the biomimetic transition layer introduced into the sample which contains the synthesized carbonate substitute calcium hydroxyapatite which when introduced into the biomimetic layer allows the molecular and chemical affinity with the anatomical dental root to be improved [5]. Due to the inclusion of the CHAP into the biomimetic transition layer in the IR-map (Fig. 2a), the interphase interface is only clearly visible between the biomimetic transition layer and dental material where a sharp colour gradation is determined by the intensity of the oscillation mode of the PO₄ CHAP group. There is no distinct native tissue/biomimetic composite interface, which is indicative of a high affinity of the latter with the enamel and dentin.

In addition to the IR-map of the phosphate group (Fig. 2a), the IR-image of the oscillations in the area 1718-1358 cm⁻¹ which belongs to the organic component of the sample was designed (Fig. 2b). This map gives a visually clearer of the enamel and dentin interface to be identified.

When it comes to the interaction of the dental material, biomimetic buffer layer and native hard tissues, it should be mentioned that the oscillation area 1718-1358 cm⁻¹ in the IR-spectrum contains a whole range of overlapping absorption bands [11], which creates certain challenges for interpreting the results and arriving at sound conclusions on the interaction in the interface area.

Analysis of the IR-maps based on the distribution of the ether molecular (–COOCH₃) (Fig. 3a) and Amide III (Fig. 3b) group which are not overlapped with the other oscillation bands in the IR-spectrum allows us to make the following conclusions on the character of the heterophase interface of the
biomimetic composite/natural hard tissue. Firstly, it is clear that the integration of the dental material with the enamel area where a shift in the intensity of the oscillation mode –COOCH$_3$ from maximum to minimum is observed in the area with the width ~14 μm and overlaps the area where the organic component dominates (Fig. 3a,b). Secondly, based on the analysis of the distribution of the absorption intensity of the Amide III group (Fig. 3b), which is found in the biomimetic transition layer, it can be concluded that the introduced biomimetic layer separates the native hard tissues from the dental material. The data obtained based on the analysis of all the IR-images (Fig. 1–3) are genuinely indicative of chemical differentiation of the functional groups of all the materials in the biomimetic system/natural hard tissue interface area and they also prove the chosen approach to analyzing the integration of dental cement new generation and biomimetic composites to be effective.

5. Conclusions
In this research has been shown the possibility of employing molecular multidimensional IR-visualization for analyzing integration of new generation biomimetic materials regenerating the mineral and organic enamel complex with native hard dental human tissues.

By means of IR-mapping of the intensity of a certain functional molecular group using synchrotron radiation the differences between a healthy tissue, dental material and biomimetic transition layer in the interphase areas were identified and visualized. The position and concentration of functional groups responsible for integration of the biomimetic composite and native hard human dental tissue was also found.

The biomimetic system we have developed based on the nanocrystal carbonate substitute hydroxyapatite obtained by means of the biogenic calcium source and the complex of major polar amino acids belonging to the organomineral human dental complex has been shown to form a functional bond with native human hard dental tissue.

The resulting microspectroscopy data is clearly indicative of the chemical differentiation of the materials and organomineral interaction at the biomimetic system/natural hard tissue interface.

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