The study of efficiency of endogenous and exogenous preventive methods of tooth enamel remineralisation by FTIR microscopy using synchrotron radiation

D L Goloshchapov 1, V M Kashkarov 1, P V Seredin 1, Y A Ippolitov 2, Y A Plotnikova 2, K Bambery 3

1Department of Solid State Physics and Nanostructures, Voronezh State University, Voronezh, Russia
2Department of Pediatric Dentistry with Orthodontia, Voronezh State Medical University, Voronezh, Russia
3Australian Synchrotron (Synchrotron Light Source Australia Pty LTD), Clayton, Victoria, Australia

Abstract. The efficiency carious preventive methods was detected with the use of equipment for IR-spectromicroscopy and high-intensive synchrotron radiation. The results of the experiment are indicative of the use of exogenous caries prevention alone (use of a toothpaste) being inadequate in saturating hard dental tissues by mineral groups and, thus, keeping teeth healthy, as this method is only short-lived. The use of endogenous methods (mineral tablets based on calcium glycerophosphate) in combination with exogenous prevention enhances prevention as part of remineralisation of dental tissues.

1. Introduction
Prevention of carious diseases is of a great importance for every human since any of cariogenic pathologies influence health and aesthetics and as a consequence social and professional activities [1]. Diagnostics of enamel at the early stages of the carious process is still a separate problem [2]. To solve it, new medical devices and techniques of analysis that assist in recognizing this disease at the earlier stages are applied [2–4]. But it goes without saying that it is better to prevent than to cure tooth decay.

A number of works made in the commercial and state-owned laboratories provide broad and diverse information on the approaches to the prevention of carious diseases. Therefore, all of the investigators assume that there are common grounds in maintaining healthy dental enamel. Their fundamental principle is hygiene of the oral cavity performed with the use of different local (exogenous) methods (tooth brushing, rinsing, applications, etc.). The second ground is based upon maintaining the microelement and organic-mineral composition of the oral fluid at the levels when remineralisation of enamel takes place, i.e. the process of its saturation with various ions and recovery of the lost mineral components [5]. The latter can be achieved by taking in pelleted mineral complex to normalize organic-mineral balance and to saturate oral fluid (endogenous methods). Certainly, oral fluid is a kind of mixed saliva containing organic components as well as inorganic ions coming both from salivary glands and from outside [6,7]. It is supposed that in case when exogenous prophylaxis is adequate and the overall health level is normal, there are no any impediments for the normal organic-mineral metabolism in oral cavity. Studies of the prophylaxis efficiency performed by the exogenous
methods, namely, tooth brushing and gels applications have demonstrated efficacy of this trend and the character of activity [8]. But it is suggested that exogenous methods of prevention do not provide the recovery of balance between the natural processes of demineralisation/ remineralisation. Due to the multi-stage character of the ion exchange remineralising agents should be retained for a long time within the oral cavity (in oral fluid) and should be in contact with the dental enamel. But in fact the time of exogenous prophylactics is less than 2 min. The presence of mineral substances in the ionized state in the oral fluid is also a necessary condition of remineralisation. This could be realized only under endogenous supply of the ions in oral fluid. Recovery of the level of natural remineralisation up to the required value depends on the long-term exposure of calcium, phosphate and fluoride ions with the optimal concentrations to the surface of enamel [6]. However, based on the experience of application and comparison of different prevention methods including fluoridation it is safe to say that the use of specimens involving phosphorus- and calcium-containing agents (for example glycerophosphate) provides the best results in the recovery of dental enamel [8–11]. Thus, all changes in the content of oral fluid can be associated with remineralisation/demineralization processes and by estimation of these changes with different kind of methods the best ways for maintaining of enamel surface can be discovered.

In our opinion, the necessity and sufficiency of the different steps in prevention can be determined by studying the changes in the composition of the oral fluid in vivo for the groups of people using different preventive agents of tooth enamel. The changes can be studied by IR-spectroscopy, which is widely used for the analysis of dental materials and for the detection of the nature of pathologies [12–14]. It allows one to obtain an extensive and various information on the phase transformations in different organic and inorganic substance, to detect changes in molecular structures of various materials. That is why this method is suitable for detecting any changes in mineral content of oral fluid. The usage of IR-synchrotron radiation increases the opportunity to detect small changes in IR-spectra with better resolution and to get spectra from micro areas with high intensity of signal.

Thus, the aim of this work is to discover the changes in the oral fluid content through the application of exogenous and endogenous preventive methods of tooth enamel remineralisation by FTIR microscopy using synchrotron radiation.

2. Materials and methods

2.1. Sampling of the materials
10 men and 10 women at the age of 18-25, European, physically healthy, without pernicious habits participated in our experiment. The regulations of making the oral fluid sampling were as follows. During the experiment and a week before the patients ate mainly vegetable food, followed a standard water consumption pattern, did not take any remedies and used no alcohol. On the eighth day after the beginning of the observations without any changes in conditions of the oral cavity hygiene in the morning just before eating, after preliminary oral rinsing patients the oral fluid was collected for the first time. This oral fluid was used as a check reference sample.

On the same day, after check sampling of the oral fluid the patients applied a tooth paste (with multi-mineral complex involving pelleted calcium glycerophosphate) for tooth brushing. Five minutes later after proceeding of the hygienic measures and a thorough oral rinsing oral fluid was sampled once again. Additionally, 30 minutes later oral fluid and once again after a thorough oral rinsing the oral fluid was sampled off the patients for the third time.

On the next day participants of the experiment just as at the first stage after eating began to take tablets (pelleted vitamin-mineral complex on the basis of calcium glycerophosphate). Participants of the group took one tablet three times a day. Three days after that, in the morning, on an empty stomach the patients thoroughly made oral rinsing and next they were again subjected to oral fluid sampling.

Each time after the sampling oral fluid was immediately centrifuged in order to remove excess water. The residue was dried at 36°C in a desiccator.
2.2. **IR-spectroscopy**

The investigations of molecular structure of the oral fluid and its chemical composition after the described sampling of specimens were performed by IR-spectroscopy. IR microspectroscopy technique was applied utilizing the equipment of the Infrared Microspectroscopy (IRM) beamline at Australian Synchrotron, Victoria, Australia. Bruker V80v Fourier transform infrared (FTIR) spectrometer and a Hyperion 3000 IR microscope (Bruker, Germany) were used to perform the analysis of microprobe of the specimens as well as a diamond high-pressure cell. Transmission IR-spectra were measured within the range of 4000 – 500 cm$^{-1}$.

3. **Results of the experiment**

The IR-spectra of the oral fluid collected from the patients, that used endogenous (use of a toothpaste) and exogenous (mineral tablets based on calcium glycerophosphate) preventive methods, are presented in Figure 1 (a),(b).

Figure 1 (a): 1 – IR-spectrum of the oral fluid taken before the experiment, 2 – spectrum of the oral fluid taken off the patients five minutes after the use of a tooth paste, 3 – spectrum of the oral fluid taken 30 minutes after the use of a tooth paste, 4 – spectrum of the used tooth paste. Figure 1 (b): 1 – IR-spectrum of the oral fluid taken before the experiment, 2 – IR-spectrum of the oral fluid taken on the fourth day after a three-day taking of the tablets, 3 – spectrum of the tablet on the basis of calcium glycerophosphate.

![Figure 1](image-url)

**Figure 1.** (a) Results from exogenous preventive methods; (b) Results from endogenous preventive methods.

From the obtained experimental data (Figure 1 (a),(b)), with comparing of literature it was confirmed that the vibration bands in the transmission IR-spectra of all the samples refer to dry residues of the oral fluids. The group of vibrations arranged in all spectra within 900 – 1200 cm$^{-1}$ is related to the P-O modes. Its appearance is connected with the presence of phosphorus derivatives in the samples: phosphates, glycerophosphates and phospholipids. This conclusion can be made by comparing the data presented in [15] where a detailed analysis of the spectra of oral fluid and human blood was performed. It is possible to confirm that the similarity of the blood and saliva spectra lead to
the fact that the modes arranged in the range of 1050 cm\(^{-1}\) refer to the organic derivatives of phosphates, glycerophosphates and phosphatase, namely, to the complex of C–O–P–O–C kind.

The next group of the vibration bands localized in the range of 1240 cm\(^{-1}\) - 1700 cm\(^{-1}\) can be related to the secondary amides: Amid I (80% C=O stretch vibr. in the range of 1615 – 1675 cm\(^{-1}\)), Amid II (60% N–H bend and 40% C–N stretch in the range of 1520 – 1575 cm\(^{-1}\)) and Amid III (40% C–N stretch, 30% N–H bend in the range of 1270-1315 cm\(^{-1}\)). Modes arranged in the experimental spectra in the range of 1400 – 1430 cm\(^{-1}\) belong to C=O str (sym) vibrations of COO and CH\(_2/CH_3\) groups. Vibration bands arranged at 500 – 700 cm\(^{-1}\) can be associated with the appearance of OCN (Amide IV), NH (Amide V) and C=O (Amide VI) vibrations of proteins.

In the IR-spectra of the oral fluid collected the fourth day (Figure 1 (b)), after three days’ taking of the tablets on the basis of a calcium glycerophosphate and the spectrum of the tablet on the basis of calcium glycerophosphate there is one more additional group of vibrations with a peak in the range of 730-770 cm\(^{-1}\). Before proceeding of the endogenous prophylaxis with the use of tablets of the mineral-vitamin complex on the basis of a calcium glycerophosphate as well as at the stage of exogenous prophylaxis using a tooth paste no any vibrations in the above-mentioned range of IR-spectrum. Based on the principles described in [16], it seems impossible to correlate a group of vibrations characterized by the maxima at 730-770 cm\(^{-1}\) with the vibrations of C-H bonds. However, this vibration mode can be related with the vibrations of P-O bond in P\(_2\)O\(_5\) group. Its appearance can be the result of decomposition of the complexes of inorganic phosphates. Arising of these vibration modes was already observed in [17] under thermal decomposition of the inorganic compounds.

The changes in organic-mineral balance of oral cavity can be explored by the calculations and analysis of mineral-organic and carbon-phosphate ratios between the mineral and organic components in the FTIR spectra of the dry residue of the oral fluid (Figure 1 (a),(b)). To calculate the first ratio it is sufficient to consider the ratio of the integral area in the IR-spectra (spectral regions at 900-1200 cm\(^{-1}\) and 730-770 cm\(^{-1}\)), to the integral area of vibration band 1615 – 1775 cm\(^{-1}\), related to Amid I (Figure 1 (a), S\(_1\)/S\(_2\); Figure 1 (b) S\(_2\)/S\(_1\)). A carbon-phosphate ratio can be calculated from the ratio of intensity of vibration bands for C=O and CH\(_2/CH_3\) bonds localized in the range of 1430 - 1400 cm\(^{-1}\) to the intensity of phosphate bands in the IR spectrum (900-1200 cm\(^{-1}\) and 730-770 cm\(^{-1}\)) (Figure 1 (a), S\(_2\)/S\(_1\); Figure 1 (b) S\(_2\)/S\(_1\)). These changes in mineral-organic and carbon-phosphate ratios are relative to the values of these ratios calculated for the reference samples at the initial stage of the investigations.

4. Discussion

The analysis of the experimental data and their comparison with the spectra of dry residues of the oral fluid demonstrated that in the IR spectra of the preventive agents one can see the groups of vibrations identical to those observed in the spectra of oral fluids (Figure 1 (a), (b)). It is due to the presence of similar organic-mineral groups and complexes in the composition of these preventive agents. Thus, application of the chosen preventive agents must be revealed in the changes of organic-mineral balance of the oral fluid and it can be observed in the IR-spectra of the investigated samples.

The analyses of the obtained data reveal that a thorough oral rinsing 5 min after tooth brushing of the oral cavity with the use of the tooth paste (involving a calcium glycerophosphate) has completely nullified its positive effect directed at the change of organic-mineral balance of the oral fluid: S\(_1\)/S\(_2\) = +7.3±2.3 S\(_2\)/S\(_1\) = -9.4±2.7. Thorough oral rinsing in 30 minutes after the application of the tooth paste for hygienic procedures of the oral cavity showed just the same effect: S\(_1\)/S\(_2\) = -1.8±2.0 S\(_2\)/S\(_1\) = -2.0±1.8. In both cases one can observe a negative relative change of the organic-mineral ratio as compared with the reference sample thus meaning washing-out of the mineral groups and complexes remaining in the oral cavity after the application of the tooth paste.

Analyzing the results of the changes of mineral-organic (S\(_2\)/S\(_1\) =+11.8±1.9) and carbon-phosphate (S\(_2\)/S\(_1\) =-53.7±1.5) ratios after taking the tablets on the basis of a calcium glycerophosphate by the patients one can make a conclusion that after taking the tablets for three days an increase in the mineral-organic ratio was observed more than 10% as compared with the reference (check) sample of the oral fluid. All of the above facts mean that organic-mineral balance in the oral fluid was shifted to
the increase in the content of the mineral groups and complexes after the application of the tablets comprising of mineral-vitamin complex on the basis of calcium glycerophosphate.

5. Conclusion
Interrelation between the oral fluid composition and efficiency of exogenous and endogenous methods of resistance to caries were shown in the work. Exogenous methods of prevention were found to provide short-term effect in maintaining organic-mineral balance of the oral fluid while endogenous ones resulted in a long-term presence of minerals and organic-mineral groups and complexes required for remineralisation of dental enamel. The organic-mineral balance in the oral fluid was shifted to the increase in the content of the mineral groups and complexes after the application of the tablets comprising of mineral complex on the basis of calcium glycerophosphate. The content of endogenous biologically accessible phosphate groups and complexes in the oral fluid, according to our data, exceeds by more than 10% that in the oral fluid before the use of the prevention agents.

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