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The investigations of changes in mineral-organic and carbon-phosphate ratios in the mixed saliva by synchrotron infrared spectroscopy



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ABSTRACT

The objective of this study was to investigate the efficiency of the saturation of mixed saliva by mineral complexes and groups necessary for the remineralisation of tooth enamel using exogenous and endogenous methods of caries prevention.

Using IR spectroscopy and high-intensity synchrotron radiation, changes in the composition of the human mixed saliva were identified when exogenous and endogenous methods of caries prevention are employed. Based on the calculations of mineral/organic and carbon/phosphate ratios, changes in the composition of the human mixed saliva depending on a certain type of prevention were identified.

It is shown that the use of a toothpaste (exogenous prevention) alone based on a multi-mineral complex including calcium glycerophosphate provides only a short-term effect of saturating the oral cavity with mineral complexes and groups. Rinsing of the oral cavity with water following the preventive use of a toothpaste completely removes the effect of the saturation of the mixed saliva with mineral groups and complexes.

The use of tablets of a multi-mineral complex with calcium glycerophosphate (endogenous prevention) in combination with exogenous prevention causes an average increase of $\sim 10\%$ in the content of mineral groups and complexes in the mixed saliva and allows long-term saturation of the oral fluid by them. This method outperforms the exogenous one owing to a long-term effect of optimal concentrations of endogenous and biologically available derivatives of phosphates on the enamel surface.

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Introduction

Cariogenic pathologies affect health and physical appearance, and thus social and professional activities. The susceptibility to caries is related to the structure and properties of the dental tissue, the structure of dentitions and jaws, diet, the composition of the mixed saliva, the quality of the performed dental prophylaxis and the level of health of the person as a whole [1–4].

It is well-known that resistance to caries is directly dependent on the composition of the mixed saliva containing organic components as well as inorganic ions coming both from salivary glands and from outside [5–9].

Modern research into caries prevention tends to make use of the general foundations of caries prevention. The first one is regular hygiene of the oral cavity using a variety of exogenous methods: brushing teeth using a toothpaste, dental floss, rinsing and

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application of special prevention tools. This not only provides mechanical cleansing of the tooth surface but also mineral substances from the exogenous tools into the oral cavity. It is thought that if exogenous prevention is duly provided, there is nothing stopping normal organic and mineral exchange from taking place between the oral cavity and enamel to restore the latter [5,10].

The second foundation is based on maintaining the microelement, organic and mineral composition of the mixed saliva so that the enamel restores naturally [11]. This becomes possible owing to the mineral components of medications, i.e., endogenous prevention. It should be noted that endogenous prevention is used in combination with exogenous prevention.

No or insufficient prevention causes caries. This kind of disease takes place under the accumulation of numerous cycles, including the processes of demineralisation and remineralisation, and takes from a month to a year [1,12,13]. The balance in the cycles is shifted towards the first of the processes (demineralisation) as a result of the vital activity of bacteria, removal of some part of the biofilm from the surface of enamel, peracidity, inadequate

organic-mineral composition of the mixed saliva and the absence of necessary preventive measures [1,2,14–16]. It has been experimentally proven that development of demineralisation is not a unidirectional destruction but rather a periodical process. It means that demineralisation in the form of destruction/dissolution of the structure motifs of enamel (nanocrystals of calcium hydroxyapatite (HAP) and its substituted forms) as a result of mechanical impact or bacteria activity [17,18] is interchanged with the stages of recovery of the hard dental tissues [4,13,19,20]. The one-time process of dental enamel recovery inverse to the demineralisation is generally named as remineralisation and is realised owing to the mixed saliva being oversaturated with ions (Ca²⁺, HPO₄²⁻, Na⁺, Mg²⁺, Cl⁻, F⁻, OH⁻, etc.) relative to the dental enamel. The essence of remineralisation is in the saturation of the upper layers of enamel with these ions and complexes, their further crystallisation, and formation of the lost mineral forms and motifs in the affected areas [21,22].

However, despite numerous approaches to finding more effective caries prevention methods [2,23,24], the interaction between the employed prevention methods, the composition of the mixed saliva and the efficiency of the saturation of the mixed saliva with ions and complexes to make remineralisation possible have not been thoroughly studied.

As was previously noted, remineralisation can be triggered artificially. This is called "remineralising therapy". It is based on the possibility of a purposeful change and maintaining the mixed saliva state (its acidity, mineral and organic composition) at a certain level when a human organism can supply all of the mineral losses [24–28]. With experience of the application and comparison of different prevention methods, including fluoridation, it is possible to ensure that the use of specimens involving phosphorus- and calcium-containing agents provides the best results in the remineralisation of dental enamel [29-35]. This in turn allows one to argue that the absence of these ions in the mixed saliva causes normalisation of exchange processes in the oral cavity. Studies of prophylaxis efficiency performed by the exogenous methods, namely tooth brushing and gel application, have demonstrated the efficacy of this trend and the character of activity [32]. However, we think that exogenous methods of prevention do not guarantee constant maintenance of the level and saturation of the mixed saliva by mineral and organic ions and complexes.

In natural conditions, mixed saliva is saturated with phosphates and calcium ions; thus, in the nearest environment of enamel prisms (the mineral component of the enamel is hydroxyapatite) there is a sufficient amount of the ions Ca²⁺, HPO₄²⁻, F⁻ and OH⁻. One should note that remineralisation of enamel is not very efficient at high concentrations of calcium ions and phosphates in the saliva or artificial calcium-containing tools [5] as these conditions cause the formation of a dental calculus. Optimal remineralisation of the caries region and artificially stimulated caries-like lesions of enamel is realised under low concentrations of calcium ions and phosphates (1–2 mmol/l), while in the demineralised region within the near-surface region of enamel these values are about 0.1 mmol/l [5].

In order to meet these conditions, remineralising agents should not only be maintained in necessary concentrations in the oral fluid but also be kept in the oral cavity for a considerable time and come in contact with the dental enamel. As was previously pointed out, a necessary condition for remineralisation is ionised mineral substances in the oral fluid, which is not very likely in exogenous ion introduction. We believe that endogenous and biologically available phosphorus and calcium derivatives can not only saturate the oral fluid with necessary mineral ions but also enhance remineralisation as there can be conditions where they can be kept in the oral fluid.

Therefore, in order to saturate the oral fluid with calcium ions and phosphorous derivatives it is promising to use calcium glycerophosphate as an active component of different preventive measures [36,37]. Different articles dealing with remineralisation of the dental enamel make an assumption about the efficiency of the use of calcium glycerophosphate [36–40]. We believe the use of endogenous prevention with calcium glycerophosphate to be capable of enhancing the saturation of the oral fluid with calcium and phosphate ions.

However, there have been no studies to identify changes in the composition of the oral fluid, its saturation with mineral ions and complexes, or the longevity of the ions enabling remineralisation of the dental enamel of the complexes if exogenous and endogenous caries prevention has not taken place. What has been established is only that prevention and remineralisation levels interact or depend on each other in ways that can be described.

Hence, the objective of our study is to investigate the efficiency of using exogenous (use of a toothpaste) and endogenous (a pelleted mineral complex based on calcium glycerophosphate) methods of caries prevention to saturate the oral fluid with mineral complexes and groups necessary for remineralisation of the dental enamel.

Materials and methods

Two hundred people (100 men and 100 women) from the age of 22–30, healthy, addiction-free, and university-educated took part in the study. All of them provided their own mixed saliva for the investigations.

During the experiment and a week prior, the patients mainly ate vegetable food, followed a standard water consumption pattern, did not take any remedies, and consumed no alcohol.

The regulations of making the mixed saliva sample were as follows (see Fig. 1).

Seven days later (after the beginning of the experiment without the changes of conditions for hygienic measures of the oral cavity), in the morning before eating, their first meal participants of the experiment sampled their own mixed saliva for the first time after oral rinsing with water. On the same day after the collection of a mixed saliva sample, the patients used the same toothpaste to brush their teeth. Five minutes later (after proceeding with the hygienic measures for the oral cavity with the use of a toothpaste and an oral rinsing with water), mixed saliva was sampled once again. Thirty minutes later (and once again after an oral rinsing for 30 s), the mixed saliva was sampled by the patients for the third time

On the next day, the participants of the experiment began to take the same tablets (a pelleted 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 carried out oral rinsing with water and they were again subjected to mixed saliva sampling.

Each time after the sampling mixed saliva was immediately centrifuged in order to remove excess water. The residue was dried at $36\,^{\circ}\text{C}$ in a desiccator.

It should be noted that in this research we have investigated mixed saliva (oral fluid) with the composition being different from that of a mix of salivary glands. For exogenous and endogenous prevention there will be derivatives of the used prevention substances in the mixed saliva which will enter it either through the oral cavity (a toothpaste) or through salivary glands (use of a mineral complex based on calcium glycerophosphate).

Investigations of the samples obtained in such a way were performed by IR spectroscopy, which is widely used for the analysis of

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