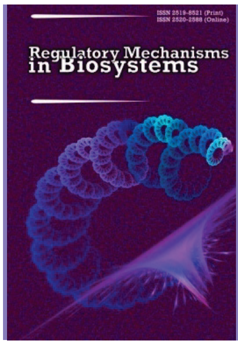

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Morphological structure of enamel caries in the dynamic process of demineralization and remineralization

N. O. Gevkaliuk, I. V. Nazarenko

Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

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Ivan Horbachevsky Ternopil National Medical University, Maydan Voli, 1, Ternopil, 46001, Ukraine.
Tel.: +38-096-560-14-45.
E-mail: gevkalyuk@tdmu.edu.ua

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According to modern ideas, the carious process at the white spot stage is reversible, so all the attention of scientists is directed to the development of means to restore the crystal lattice of tooth enamel. The purpose of the study is to evaluate morphological changes in caries at the white spot stage and the process of structural remineralization of the carious lesion area. Clinically active initial lesions were detected visually, using optical magnification and positive staining. Structural changes in enamel were studied by scanning electron microscopy of surface impressions obtained from the surface of demineralized enamel. Remineralizing therapy was carried out by applying ApaCare restorative gel (Germany) with nanodispersed highly active hydroxyapatite. Determination of the intensity of dental caries by the DMFT and dmft index showed 9.04 ± 0.40 points on average in the group of subjects, children with code 1 according to the ICDAS index made up 50.8%. Colour reactions showed 7.80 ± 0.21 points on average in the group. SEM visualized characteristic structural changes in the area of the carious spot – an increase in the porosity of the enamel surface with exposure of the fine crystalline structure and destruction of interprismatic fibrillar structures. When using a remineralizing agent, a roller-like deposition of an amorphous remineralizing substance is characteristic, and at the final stage, a smooth enamel surface with a fine crystal lattice is restored. The restoration of the prismatic structure of the enamel occurs due to the mineralization of the preserved fibrillar walls in the interprismatic space. Clinically, at this stage, the enamel visually restores its original shine, smoothness, and colour. In 81.8% of cases, the dye did not penetrate the previously demineralized enamel. The high level of non-cavitated active caries lesions can be largely controlled by the use of remineralizing agents. Further research involves studying the quantitative characteristics of the processes of de- and remineralization of tooth enamel.

Keywords: tooth enamel caries; white spots; tooth demineralization diagnostics; violation of mineralization; re-mineralizing preparations; nanodispersed hydroxyapatite; SEM and caries morphology.

Introduction

Today, the prevention and treatment of dental caries in children remain an important and relevant problem of modern practical dentistry that attracts the special attention of children's dentists (Anil & Anand, 2017; Giacaman et al., 2018; BaniHani et al., 2022). Research by a number of authors indicates that in recent years there has been a tendency to increase in the prevalence and intensity of dental caries in children (Dorri et al., 2015; Garcia et al., 2021). The fact that the number of young children with caries-affected teeth has increased by almost three times is alarming. Moreover, a number of studies indicate that such a trend may be associated with an increase in the frequency of pregnancy pathology, a genetic predisposition to the incidence of dental caries, a worsening of the environmental situation, the result of which is a decrease in the overall resistance of the child's body (Anil & Anand, 2017; Abdelrahman et al., 2021; Hasiuk et al., 2021). It is known that the prevalence of dental caries in early childhood varies from 20% to 80% in different regions, and 20% of 6-year-old children are already diagnosed with caries of permanent teeth (Bottner et al., 2020; Lamba et al., 2020). According to data from the World Health Assembly (Monajem, 2009), from 20% to 90% of 6-year-old children have dental caries, and by the time they reach 12 years of age, they already have an average of 0.5 to 3.5 permanent teeth affected. According to the WHO (Petersen & Lennon, 2004), about 60–90% of school-age children suffer from dental caries.

According to modern ideas, dental caries is the most common pathological process that occurs under the influence of etiological factors under certain pathogenetic conditions and is characterized by focal demineralization of hard tooth tissues with the formation of a carious cavity (Farooqi et al., 2015; Fontana & Gonzalez-Cabezas, 2019). The use of different approaches to remineralization therapy to restore the crystalline lattice of tooth enamel is characterized by variability in the effectiveness of prevention of dental caries (Brunton et al., 2013; Krois et al., 2018; Flynn et al., 2022). It is known that the prevention and treatment of initial dental caries are effective when combining treatment and prevention measures. These measures include rational oral hygiene, the use of exogenous and endogenous preventive measures – the use of fluorides, calcium preparations, vitamins, herbal preparations, dietary supplements, local remineralizing therapy, etc. (Amaechi, 2015; Usković, 2015). It is known that at the white spot stage, the carious process is reversed, so all the attention of scientists is directed to the development of means to restore the crystalline lattice of tooth enamel (Greenwall, 2013; Kamina, 2013; Han et al., 2017).

The aim of the study was to evaluate the morphological changes during the development of the carious process in the white spot stage and the process of structural remineralization of the carious lesion zone using scanning electron microscopy (SEM).

Materials and methods

All the research methods which were used met the requirements for examination of patients and the scientific researches conducted met the requirements of norms and principles of bioethics. In carrying out the work, the rules of patient safety, the rights and canons of human dignity, as well as the moral and ethical norms in accordance to the main conditions of the GSP were followed (1996); European Convention on Human

Rights and Biomedicine (dated 04.04.1997); a set of ethical principles regarding human experimentation developed for the medical community by the World Medical Association (WMA) – The Declaration of Helsinki (2013); the Order of the Ministry of Health of Ukraine No 281 (dated November 1, 2000); a code of ethics for scientists of Ukraine (2009). This is confirmed by the minutes of the Bioethics Commission of the Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (11.01.2021, No. 62). The study design was approved by the Bioethics Committee. Violations of moral and ethical norms during the conduct of research work were not detected.

The study was conducted at the dental department of the University Hospital in Ternopil. We examined 79 children aged 8 to 12 years, among whom the proportion of children with initial dental caries was 87.8% (65 people), who were included in the study groups. Prior to the study, children and their parents were explained the purpose of the study. Initial examination and medical check-up of children were carried out subject to written informed consent of patients (parents of the children). The following factors were taken into account for participation in clinical trials of children: a) informed parental consent for research; b) verified clinical diagnosis (ICDAS code 1).

Clinical examination of the oral cavity was performed according to the standard scheme. The main clinical method for detecting carious lesions was visual examination of the teeth, which allows one to diagnose carious defects of hard tissues visible to the naked eye at the initial stages of their development. Visual and tactile examination was performed using a sharp explorer. To assess carious changes in the enamel, indices based on the International Caries Detection and Assessment System (ICDAS) codes were used (Pitts & Ekstrand, 2013). The study included children with the initial stage of carious lesions, in which the first changes in enamel visible after drying the tooth were visualized (ICDAS code 1). For visual examination of the teeth, an instrumental method was used – an optical magnification device (camera). After a hygienic rinse of the mouth with a solution of mouthwash – Dentaid Perio-Aid Intensive Care (Spain), professional dental hygiene was performed.

To detect foci of enamel demineralization, vital staining with Caries Marker (Voco) was used. A special solution of caries marker was applied to the vestibular surface of the anterior teeth directly from a dropper for 10 seconds, after which it was rinsed with water. When the drug is applied to the affected enamel, its colour turns blue, which makes it possible to determine the development of the disease at the stage of the stain with the definition of its boundaries. The method of colour reactions with a 2% aqueous solution of methylene blue was used to determine the intensity of staining. The method is based on the ability of the dye to penetrate demineralized enamel and thus stain a carious spot (the dye does not penetrate intact enamel). The intensity of the staining shade was interpreted using a 10-point Axamit scale. For the visual diagnosis of enamel caries, we used the criteria developed by K. Ekstrand (Fontana & Gonzalez-Cabezas, 2019), according to which the appearance of a carious lesion can be used to suggest the depth of demineralization of tooth tissues. Remineralization therapy was performed by applying ApaCare "Liquid enamel" restorative gel (Germany) according to the manufacturer's recommendations. For oral hygiene, a hygienic toothpaste (of the patient's choice) was recommended, depending on the age group of the child. It was recommended to use a toothbrush with soft (with a fiber diameter of up to 0.2 mm) or extra soft (with a fiber diameter of 0.15–0.18 mm) nylon bristles to prevent mechanical damage to the fibrillar of the enamel matrix in the area of the carious spot.

Morphological studies were carried out in the interdepartmental educational and scientific laboratory of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine. We randomly selected 45 replica samples to evaluate the changes in the enamel surface by SEM during the examination before and after remineralization. Structural changes in the enamel of permanent teeth in children aged 8–12 years were studied using scanning electron microscopy (SEM) (PEM-102-E, VO "SELMI", Sumy, Ukraine) of surface casts-imprints. It allows you to study the surface of the enamel multiple times without removing the tooth affected by caries. This is especially relevant when there is a need to study the dynamics of remineralization processes. They were obtained with the help of a 1% aqueous solution of polyvinyl alcohol, which was sprayed with a thin layer of chemically pure aluminum (sample 999) followed by shading with chromium sprayed in a VUP-6M magnetron attachment (Selmi, Sumy, Ukraine). Cast-imprints were mounted on metal tables with the help of conductive glue, the "working" surface of which was directed towards the incident electron beam, with the possibility of reorientation at different angles (15–75°). This enables a better visualization of the surface structures on the mold-impression, when the enamel surface is locally shaded by the protruding elements of the microrelief. The drugs were examined in reflected electron mode with an accelerating voltage of 15–20 kV. Micrographs of the areas selected for illustration were taken by displaying them on a computer monitor using a Vision Color CCD Camera.

Statistical analysis of research results was carried out using Statistica for Windows (Version 8). Descriptive statistics included the calculation of relative and average values. Categorical signs are presented in the form of relative indicators (percentage of patients with the presence of the sign in the group). Quantitative indicators were presented in the form of $x \pm SE$ (mean ± standard error).

Results

Determination of the intensity of dental caries according to the DMFT and dmft indexes showed that on average, the caries intensity in the group of subjects was 9.04 ± 0.40 points, with the average value of the caries intensity index of permanent teeth being 4.11 ± 0.40 points. Out of the total number of examined patients with dental caries (65 people), children with ICDAS code 1 accounted for 50.8% (33 people). The average number of surfaces with non-cavitated lesions in these children was 4.64 ± 0.51. When examining the condition of the hard tissues of the teeth in the cervical areas of the anterior group, whitish spots with clear contours and loss of normal enamel luster were determined. When probing the foci of demineralization, enamel rustling and sensitivity in the affected area were detected. Sometimes patients complained of a sensation of cramping when eating spicy or sour foods. The method of colour reactions showed that when stained with a 2% methylene blue solution, carious spots were coloured on average in the group of subjects in a dark blue colour – 7.80 ± 0.21 points. The high level of non-cavitated lesions of active caries indicates that caries in this population can be largely controlled by preventive measures.

Preliminary removal of bacterial plaque from the surface of the enamel in the area of the carious spot allows visualization of characteristic SEM patterns of local demineralization (Fig. 1). These patterns have a rosette-like shape of the "crow's foot" type (Fig. 1a) and indicate structural changes in the enamel in the area of the carious spot. They are manifested by an increase in the porosity of its surface with the exposure of a fine crystalline structure at the bottom of the demineralization zone in the "fish scale" type (Fig. 1b). In general, the SEM patterns of demineralization are first caused by the demineralization of the core, and then the core of the enamel prism with the exposure of the fibrillar interprismatic framework of the honeycomb structure (Fig. 1c). This frame has increased adhesive ability to both organic and inorganic material. The preserved fibrillar matrix allows the remineralization agent to penetrate deeper and firmly fix in the damaged enamel. This explains and justifies the need to preserve the fibrillar interprismatic framework as the structural basis of the remineralization process.

The loss of enamel surface smoothness occurs under the influence of excessive bacterial contamination of the oral cavity, the accumulation of their waste products, which plays the role of an exposure factor in the occurrence of caries. Due to the acidic environment of the bacterial flora in the dental plaque, bacterial acid etching of healthy enamel occurs, which creates the basis for increasing the retention capacity of its surface and increasing the total surface area by increasing the microrelief of the enamel. It is possible to completely remove the reacted inert enamel decay products, which leads to the exposure of the fibrillar surface of the enamel, which is more capable of remineralization. One of the methods of treatment of the initial forms of dental caries – remineralizing therapy – is to close the enamel pores for the penetration of acids and the release of dissolved minerals from the surface of the enamel. The process of remineralization of tooth enamel consists of two stages: 1) initial, unfinished

(Fig. 2a, 2b); and 2) final, completed remineralization (Fig. 2c, 2d). Each of these stages has its own SEM pattern. The first stage is characterized by a roller-like layering of amorphous remineralizing substance. As a rule, this process is found on the periphery of the carious spot, and its structural essence is manifested by the "sealing" of focal pits and the cellular enamel matrix. For the second stage, the structural essence is the restoration of a smooth enamel surface with a fine-crystalline lattice lattice protruding from the middle (Fig. 3). We have shown that the surface zone is an active zone compared to an intact unaffected area of enamel covering the subsurface layer of the lesion (Fig. 4). The superficial zone is the first zone that is most capable of remineralizing therapy, and when the remineralizing agent is applied, new SEM patterns of remineralization are created (Fig. 4). At the same time, the remineralizing drug has specific ways of deposition on the demineralized enamel surface, which are similar to those described for acid-etched healthy enamel (Fig. 5). Applying a remineralizing agent for therapeutic purposes to the structurally altered carious enamel surface and the adjacent healthy enamel can effectively isolate the affected area and prevent further progression of the carious process.

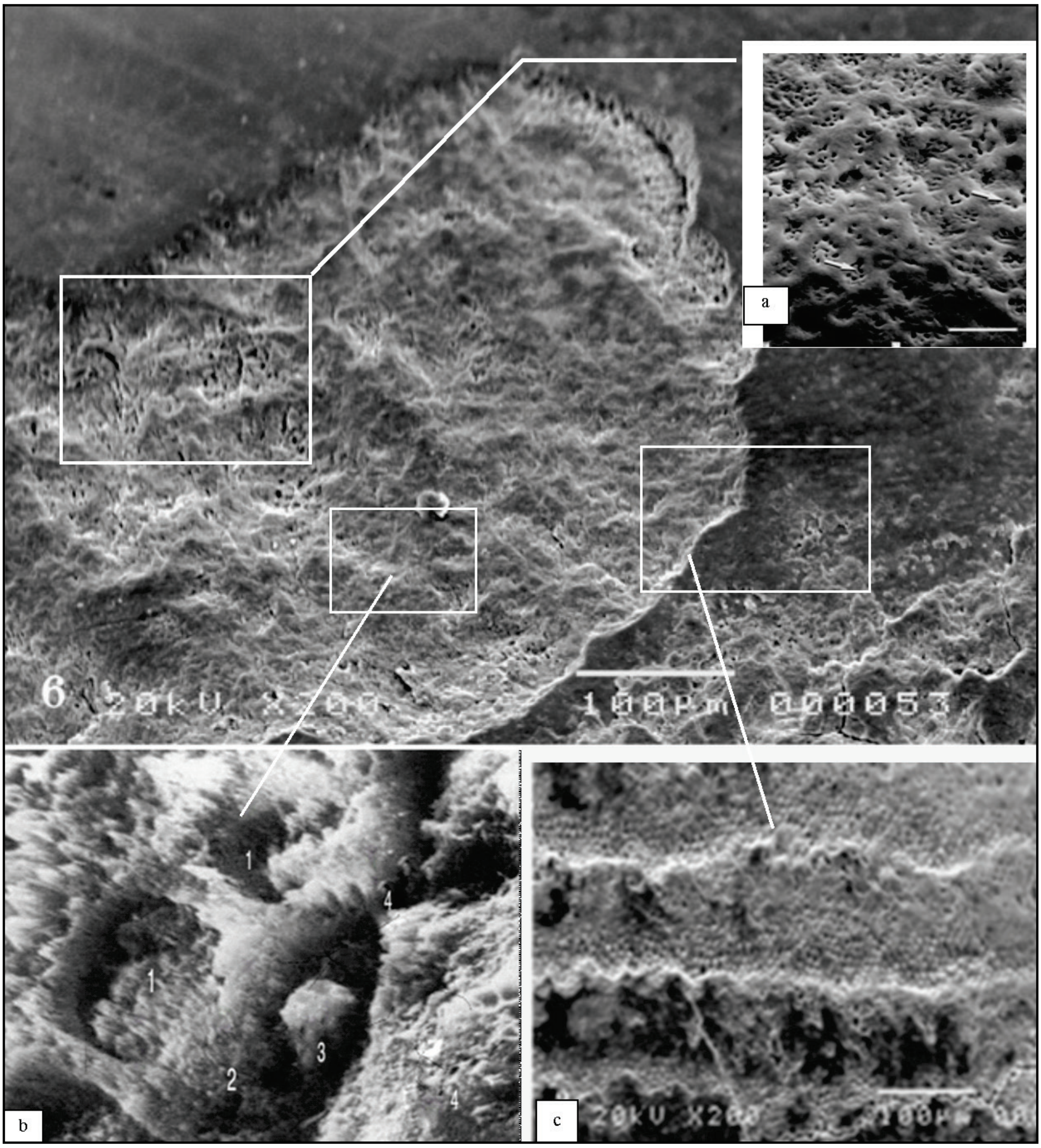

Fig. 1. Structural organization of the enamel surface in the carious spot zone: *a* – a general view of the enamel demineralization zone with a rosette-like "crow's foot" SEM pattern (arrows); *b* – demineralization zone of the "fish scale" type with a characteristic fine crystal structure at the bottom of the enamel demineralization zone: *1* – destructuring of the nucleus of the enamel prism; *2* – the fibrillar framework of the enamel prism; *3* – remains of the crystalline structure in the interprismatic space; *4* – thickening of the fibrillar structure in the interprismatic space; *c* – cellular SEM pattern formed due to the destructuring of the core and nucleus of the enamel prism; scanning electron microscopy

In addition, it makes it possible to create an intact, slightly porous surface zone with the help of remineralization therapy and thereby promote further remineralization of the damaged enamel zone. This happens through repeated sessions of remineralizing therapy, in which each subsequent layer of remineralizing drug is mechanically superimposed on the framework already created as a result of the previous session of remineralizing therapy (Fig. 6). Repeated sessions of remineralizing therapy make it possible to observe the precipitation of crystals on the surface of demineralized enamel. The data obtained indicate the formation of globular structures of various sizes, restoration of interprismatic structures, nuclei and cores of enamel prisms. SEM patterns of demineralization completely disappear: the size of defects and the number of focal microcavities and pits on the surface decrease, so the enamel has a smooth appearance (Fig. 7). During the dispensary observation, no increase in the intensity of dental caries was noted in the examined children, and there were no new foci of enamel demineralization. Clinically, at this stage, the enamel looks visually intact, has an initial shine, smoothness, colour without pigmentation and does not differ in shade from the surrounding healthy enamel (Fig. 8).

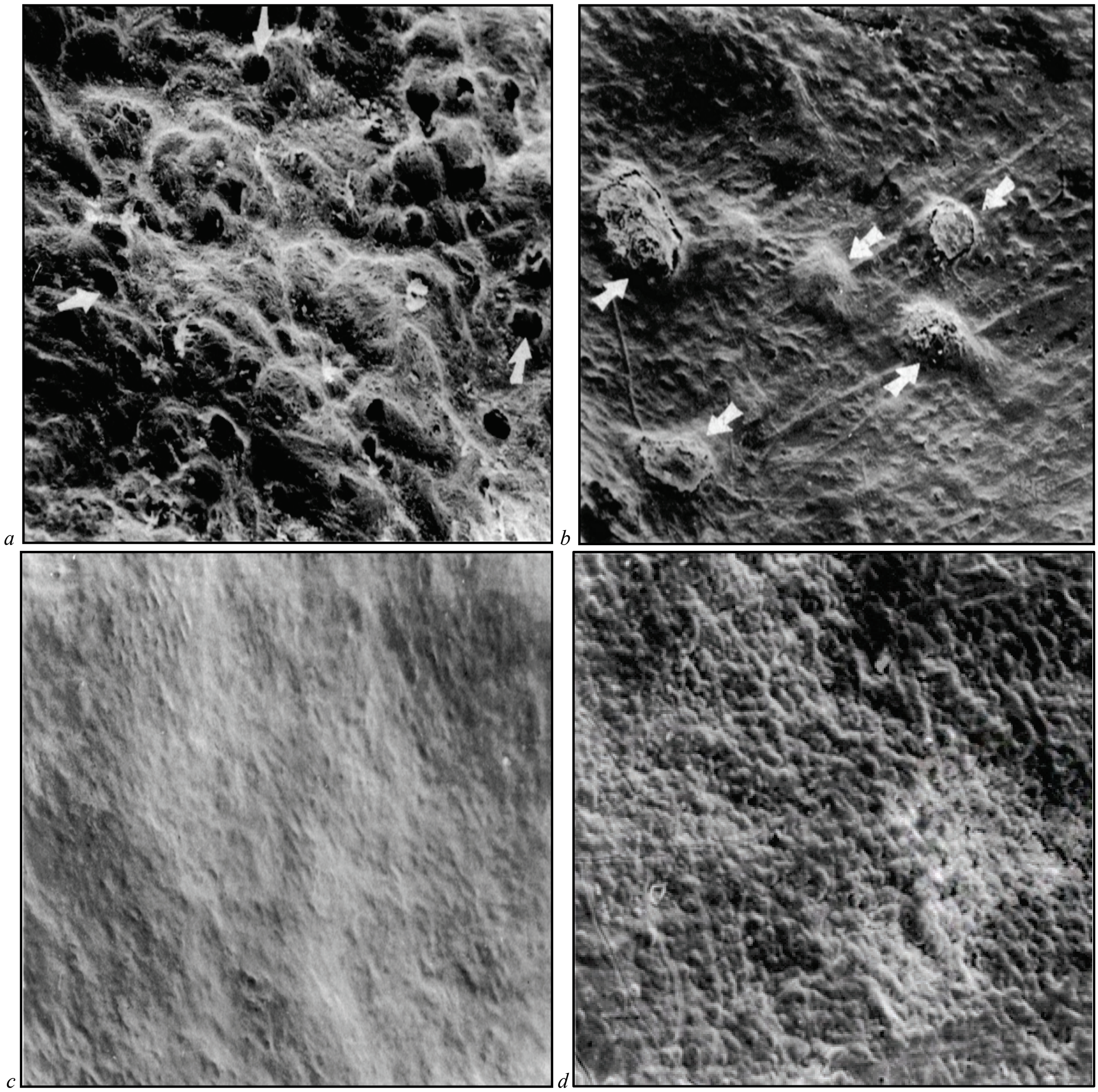

Fig. 2. Morphological changes or structural substrate of different stages of enamel remineralization in the carious spot zone: restoration of a smooth enamel surface (*a*, *b*) and remineralization "sealing" of focal pits of demineralization at the bottom of a carious spot (arrows) after application of a remineralizing drug (*c*, *d*); scanning electron microscopy

During the observation period, there were no complaints of ossification in the patients' anamnesis. White spots in the cervical areas of the teeth were not clinically detected in 27 of the examined children and were barely noticeable in 6 of the examined children, which was further confirmed by the method of colour reactions. Clinically, in the areas of previously demineralized enamel, the restoration of the natural gloss and colour characteristic of intact enamel was observed. Sensitivity and rustling of the enamel in the affected area were not detected during probing, the surface texture of the perifocal areas of the enamel was homogeneous. A positive probe sliding symptom was observed, and the probe tip revealed a characteristic natural sound resonance. The method of colour reactions with a 2% aqueous solution of methylene blue showed that the dye did not penetrate (0 points) into previously demineralized enamel after remineralizing therapy in 81.8% of cases and barely stained (1 point) in 18.2% of cases. The data obtained testify in favour of restoring the functional integrity of the enamel in the vast majority of the examined patients.

Discussion

Today, the primary focus of dentists is to prevent demineralization of tooth enamel to avoid the possible development of a white spot or cavitated lesion over time. The key to the prevention of dental caries is the modulation of the balance of demineralization and remineralization processes. Decades of research have led to the development of technologies that can reduce enamel demineralization and promote tooth enamel remineralization. Today, there are many alternative approaches to remineralization of tooth enamel that provide convincing evidence of the effectiveness of remineralization and reduction of white spots in initial dental caries. Additional clinical studies in vivo to confirm the effectiveness of remineralizing therapy remain relevant. In order to develop effective enamel remineralization mechanisms, it is important to understand the process of demineralization and remineralization and the ways in which this balance is affected.

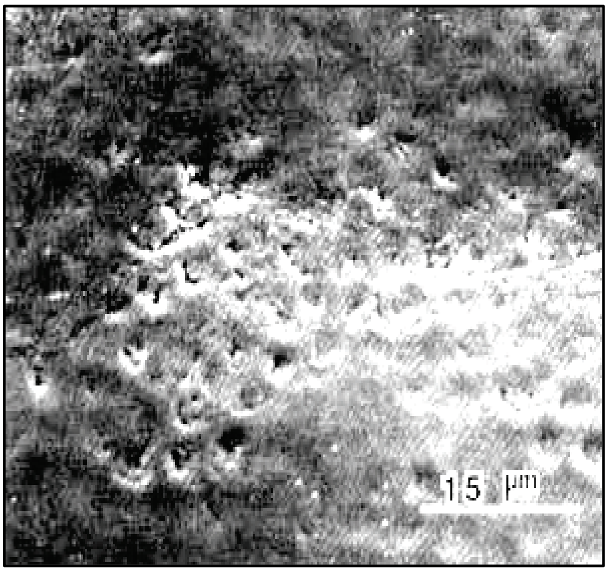

Fig. 3. Structural organization of enamel with enriched microrelief at the stage of incomplete remineralization; scanning electron microscopy

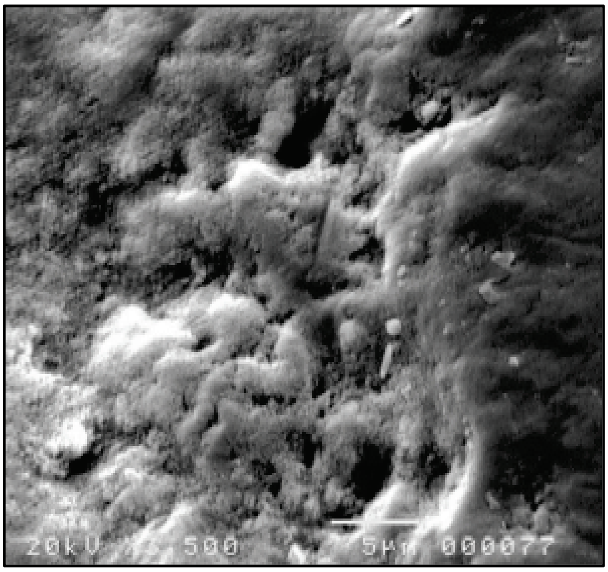

Fig. 4. Spherical crystals of the remineralizing agent settle in layers on the demineralized enamel surface; scanning electron microscopy

The role of oral fluid in the processes of de- and remineralization. Ion exchange processes that occur in the hard tissues of the tooth are largely determined by the properties of the oral fluid in contact with the enamel surface. Changes in the level of permeability of enamel under physiological conditions occur mainly due to the chemical influence of oral fluid (Ramezani et al., 2021). Human saliva contains a number of physical, physico-chemical and chemical agents that protect the tissues of the oral cavity from chemical compounds, in particular those produced by various microorganisms (Díaz-Garrido et al., 2020; Ramezani et al., 2021). Among the protective factors of saliva, the effective removal of microorganisms and their waste products is important, which is facilitated by constant moistening of the oral cavity, which guarantees the constant presence of both non-immune and immune factors (Kumar et al., 2017). Saliva contributes to the biomineralization of teeth in the oral cavity due to the presence of soluble minerals in it. Enamel is permeable in both directions, the degree of its permeability is not the same in different age periods and decreases with the development of the tooth. Enamel does not contain cells and is not able to regenerate in case of damage, but it constantly undergoes metabolism (Kumar et al., 2017). Healthy enamel contains about 4% free water, which fills the spaces in the crystal lattice and organic matrix. These spaces make up 6–12% of the total volume of enamel. It is believed that such properties of enamel as permeability and solubility depend on the amount of free water and its volume. Actually, these properties of enamel are the basis of the processes of de- and remineralization. Violation of the dynamic balance in the mechanism of mineral exchange in enamel leads to the development of dental caries (Rathnayake et al., 2013; Pedersen et al., 2018).

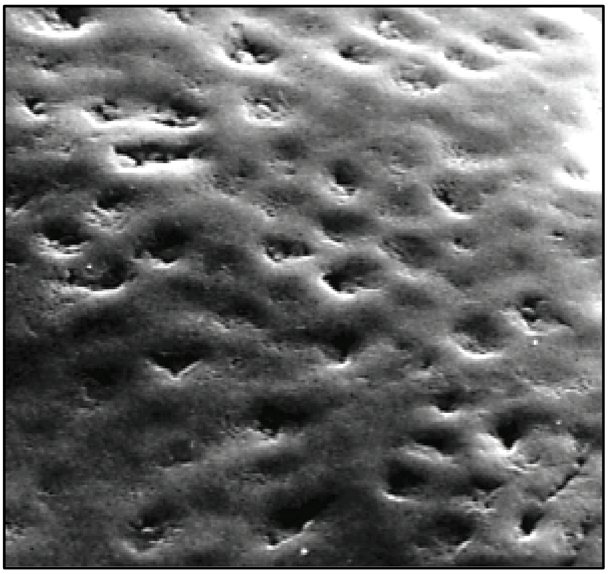

Fig. 5. Enamel structure at the stage of incomplete remineralization: scanning electron microscopy; magnification: × 3500

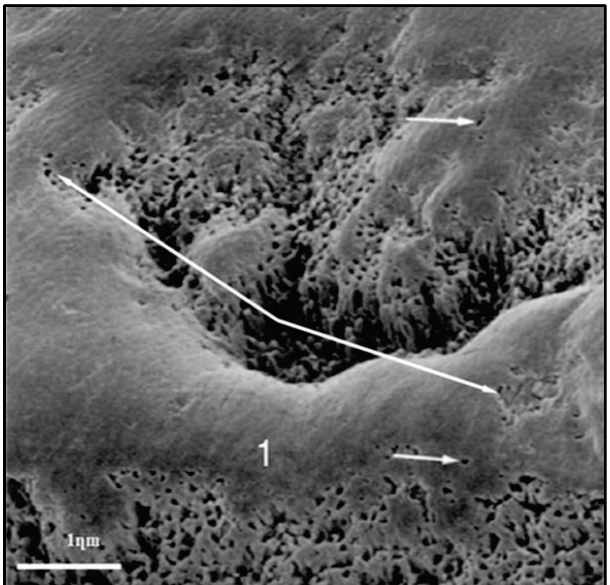

Fig. 6. The roller-like structure (*1*) of the remineralizing substance, which is deposited in "flows" in the zone of enamel with increased wear and on its surface also has zones of increased retention (arrows); scanning electron microscopy

Structural and functional structure of enamel. Enamel consists of enamel prisms, interprism substance and is covered with a cuticle. Enamel prisms are the main structural and functional units of enamel, the diameter of enamel prisms is 3–5 μm, increasing from the enamel-dentine junction to the enamel surface. They pass in bundles through the entire thickness of the enamel, located mainly perpendicular to the enamel-dentine junction (Desoutter et al., 2023). Enamel prisms are characterized by alternating light and dark stripes with an interval of 4 μm. It is believed that the dark and light areas of the enamel prism have different levels of mineralization. The most peripheral part of each prism is the prism shell – it is part of the prism itself, a narrow layer of low-mineralized substance containing a large amount of protein (Somasundaram et al., 2013). The organic matrix associated with the crystals, during the formation of enamel, ensures the processes of their growth and orientation (Lacruz et al., 2017).

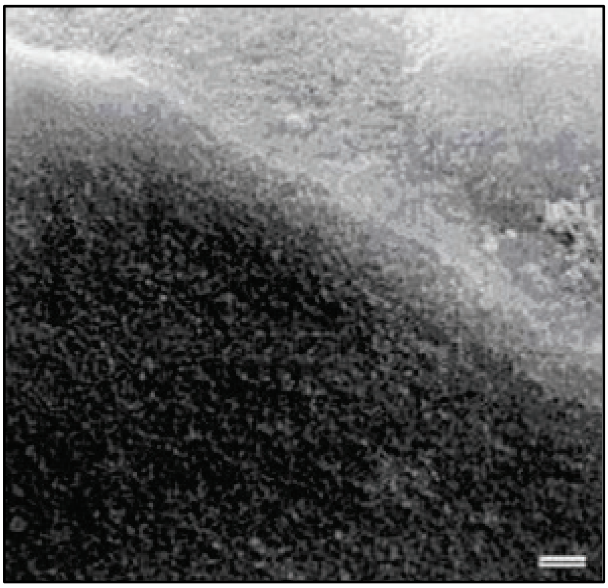

Fig. 7. General appearance of tooth enamel after completion of remineralization therapy sessions; scanning electron microscopy

a

b

Fig. 8. Active initial lesions of tooth enamel (non-cavitated) before (*a*) and after remineralization (*b*)

Enamel prisms are surrounded by an interprism substance, the thickness of which is very small – it does not exceed 1 µm. The degree of mineralization of the interprism substance is lower than that of enamel prisms. In view of this, enamel demineralization occurs in the following sequence: first in the area of enamel prism shells, later – in the interprism substance, and only then in the prisms themselves (Tsai et al., 2019). The inorganic material – the layer of tooth enamel – is extremely strong, mainly consisting of calcium hydroxyapatite crystals (Pajor et al., 2019). However, hydroxyapatite is prone to dissolution under the influence of acids, which in the presence of cariogenic bacteria can lead to the development of caries (Erdem et al., 2013; Díaz-Garrido et al., 2020; Liu et al., 2023). In response to the influence of acids, waste products of microorganisms, hydroxyapatite dissolves, while calcium is removed from the tooth structure, leaving it demineralized (Abou Neel et al., 2016). With constant demineralization of the enamel surface, a significant amount of minerals is lost, so the enamel cannot remineralize solely due to the presence of calcium hydroxyapatite, which is contained in the oral fluid. The presence of fluoride in the oral fluid makes the tooth structure stable due to the formation of a changed crystal in the enamel – calcium fluorapatite instead of calcium hydroxyapatite. Calcium fluorapatite is more difficult to dissolve and does not begin to demineralize until pH 4.5, while calcium hydroxyapatite has a critical pH of 5.5. Demineralization occurs when the pH of the oral environment drops below 5.5, allowing calcium and phosphate ions to diffuse from the enamel surface (Shen et al., 2015; Abou Neel et al., 2016). The duration and frequency of acid challenge can affect the level of damage to the tooth structure, which can range from small white spots to cavitation (Rirattanapong et al., 2017). It should be emphasized that when the enamel surface remains partially intact, there is an opportunity to stop carious lesions by the joint action of saliva minerals and fluoride therapy (Vanichvatana & Auychai, 2013; Farooq & Bugshan, 2020).

Promotion of remineralization of early enamel damage. For many years, the remineralization of tooth enamel has remained the main area of research, since it is still not possible to accurately determine the effectiveness of various methods of remineralization. To this day, fluorotherapy remains the most popular of all existing methods of dental caries prevention and remineralization (Crescente et al., 2022). The use of fluoride is an effective method of promoting remineralization of early enamel damage due to the formation of fluorapatite. However, for every two fluoride ions, ten calcium ions and six phosphate ions are required to form one unit cell of fluorapatite – $Ca_{10}(PO_4)_6F_2$. Therefore, with local application of fluoride, an insufficient amount of available calcium and phosphate ions can limit the enamel remineralization process (Grohe & Mittler, 2021; Jafarzadeh et al., 2022). Toothpaste with fluoride can be more effective for enhancing remineralization, if tricalcium phosphate is added to it. It has been established that this agent delays the interaction of calcium with ionic fluoride, which allows more fluoride and calcium ions to be delivered to the demineralized enamel (Vanicvatana & Auychai, 2013; Rirattanapong et al., 2017). It should be noted that although fluoride ions are adsorbed on the surface of enamel crystals, preventing dissolution and enhancing remineralization, demineralized surface lesions remineralize faster and more fully than subsurface lesions. Khoroushi & Kachuie (2017) have cautioned against treating white spots after orthodontic treatment with concentrated fluoride agents, as the remineralization procedure would stop enamel damage and prevent complete restoration by hypermineralization of the surface.

Therefore, the use of fluoride alone as a remineralizing agent in clinical practice is ambiguous. As for non-fluoride therapy, today there are many agents capable of remineralization. Recently, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has become popular as a derivative of milk protein casein. CPP stabilizes calcium phosphate, making it more soluble and biologically available in dental biofilms. The advantage of bioavailable calcium and phosphate is to normalize intra-oral pH, which reduces the frequency of enamel demineralization (Reema et al., 2014; Gupta et al., 2016). A comparative evaluation of the remineralization potential of casein phosphopeptide-amorphous calcium phosphate and casein phosphopeptide-amorphous calcium phosphate fluoride on an artificial enamel white spot lesion by Mehta et al. (2013) demonstrated a clear improvement in remineralization and reduction of white spots. As an alternative to fluoride, the use of nanohydroxyapatite, which is a biocompatible and bioactive medium, is promising for remineralization. It is well suited for remineralization of enamel even in neutral pH conditions because it has a structure similar to apatite crystals in enamel (Grohe & Mittler, 2021). The use of calcium-sodium-phosphosilicate bioglass is promising in enamel remineralization, the effect of which is provided by the release of calcium and phosphate ions (Grohe & Mittler, 2021). Research by Wagh (2013) shows that calcium-sodium-phosphosilicate bioglass can enhance remineralization through the formation of apatite (Dhanya et al., 2021). However, more in vivo studies are needed to confirm efficacy before recommending it as an alternative to fluoride. A new strategy of remineralization therapy is the use of oral probiotics. Although probiotics do not directly affect enamel remineralization, some studies show that they may promote this process by affecting oral pH and increasing the likelihood of remineralization by using available minerals in saliva (Cagetti et al., 2013; Sivamaruthi et al., 2020). The specific mechanism of action of probiotics in the process of remineralization is not fully understood, and the available evidence is contradictory (Sivamaruthi et al., 2020).

Nanotechnological strategies for tooth enamel remineralization. Treatment of non-cavitated lesions should be aimed at stopping lesion progression and improving aesthetics by reducing opacity (Warreth, 2023). Nanotechnological strategies for the remineralization of tooth enamel began to be applied relatively recently. Today, clinicians prefer bioactive materials that correspond to the current practical philosophy of using remineralization agents and minimally invasive therapy (Cheng et al., 2015; Anil et al., 2022; Zhang al., 2023). Nanotechnology holds the promise of inhibiting caries by controlling biofilm acids and enhancing remineralization. That is why we used ApaCare & Repair teeth restoration gel in our study to remineralize white spots on children's tooth enamel. In its composition, it contains 7.0% of nanodispersed highly active hydroxyapatite, which restores damaged and carious tooth enamel and prevents demineralization. In addition, ApaCare & Repair normalizes salivation, promotes the maturation of tooth enamel in young children, especially those with a high tendency to tooth decay. Our study included children with initial enamel caries without macroscopic signs of dental tissue destruction. It is known that enamel demineralization can be detected by invasive and non-invasive methods. One of the available non-invasive diagnostic methods is SEM, as white spots cannot be detected visually until they penetrate the enamel by 200–300 µm (Alghilan et al., 2019). Studies conducted by Soares et al. (2017), using scanning electron microscopy to assess enamel remineralization after treatment with four different remineralizing agents, showed that the self-assembling peptide P 11-4 was more effective in remineralizing enamel lesions compared to other investigated agents. The SEM micrographs obtained by the authors of the studied groups showed either amorphous crystals, or particles scattered on the surface, or lines of remineralization along the prismatic boundaries. Dhanya et al. (2021), comparing NovaMin™ and CPP-ACPF using DIAGNOdent and SEM, showed that CPP-ACPF demonstrated a higher level of remineralization than NovaMin™, but both products have the potential for remineralization.

SEM photomicrographs demonstrated structural pathways from the enamel surface to the subsurface lesion. The SEM pattern in the area of the carious spot allows one to visualize the local demineralization of the rosette shape, which is characterized by an increase in the porosity of its surface with the exposure of a fine crystalline structure at the bottom of the demineralization zone. Structural changes included increased prism junctions, diffuse destruction of minerals in prism cores, and destruction of interprismatic matter. There are numerous small, rounded recesses due to the dissolved tops of the prisms. The shells of the prisms are mainly demineralized, and later the demineralization of the cores of the prisms is observed. Our SEM studies of surface casts-imprints allow us to study the surface of the enamel multiple times without removing the tooth affected by caries, which is especially important when studying the dynamics of remineralization processes. The SEM pattern at the first stage was characterized by a roller-like layering of amorphous remineralizing substance. At the second stage, the restoration of the structure of the enamel surface with a fine crystal lattice protruding from the middle was observed. On the surface of the lesions, signs of remineralization of the initial carious lesions are visible, which contradicts the concept of an intact surface layer of enamel. Clinically, at this stage, the enamel looks visually intact, has an initial shine, smoothness, colour without pigmentation and does not differ in shade from the surrounding healthy enamel. Research using high-resolution SEM demonstrated morphological changes of crystals, their shape and orientation during the formation of a carious lesion and in the process of enamel remineralization. Taking into account the importance of the surface layer in the progression of caries, the assessment of structural changes in this area is important for studying the processes of enamel de- and remineralization.

Conclusions

Determination of the intensity of dental caries by the DMFT and dmft index showed 9.04 ± 0.40 points on average in the group of subjects, children with code 1 according to the ICDAS index made up 50.8%. The index of colour reactions averaged 7.80 ± 0.21 points in the group. SEM visualized characteristic structural changes in the area of the carious spot – an increase in the porosity of the enamel surface with exposure of a fine crystalline structure at the bottom of the demineralization zone and destruction of interprismatic fibrillar structures. Remineralizing therapy with ApaCare gel with nanodispersed highly active hydroxyapatite showed that during the observation period, white spots on the tooth enamel were clinically detected only in 18.2% of cases, but they were barely noticeable (1 point) by the colour reaction method. In 81.8% of cases, the dye did not penetrate the previously demineralized enamel. The use of nanohydroxyapatite in SEM showed the precipitation of crystals on the surface of demineralized enamel, indicating the formation of globular structures of various sizes, the restoration of interprismatic structures, nuclei and cores of enamel prisms. The size of the defects and the number of focal microcavities and pits on the enamel surface also decreased. SEM patterns of enamel demineralization completely disappeared. A significant proportion of non-carious lesions of active caries in children prompts the implementation of therapeutic and preventive measures, including the use of remineralizing agents. Further research involves a comparative characterization of various remineralizing agents and the study of the quantitative characteristics of the processes of de- and remineralization of tooth enamel.

The authors declare no conflict of interest.

Written informed consent to participate in the study was obtained from all patients (parents of children). Written informed consent was obtained from patients (parents of children) for the publication of this article.

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