Efficiency of dental caries prevention with the use of polymer-based toothpastes modified by the electromagnetic field

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Abstract

The most important problems of modern dentistry cover not only the early diagnosis but also prevention of dental caries, including the one based on the use of various hygienic products and therapeutic agents. Normally, the processes of re- and demineralization in the oral cavity are in a state of dynamic. However, in the presence of cariogenic factors, the balance is shifted towards demineralization. In the event of initial caries, enamel remineralization is possible only to a certain extent, and specifically, for a reversible degree of carious lesion. There was an idea about structural changes in polymer-based consumable dental materials including toothpastes. The purpose of the study is to increase the efficiency of dental caries prevention based on the activation of metabolic processes in dental hard tissues with the use of R.O.C.S. Active Calcium toothpastes (Russian-Swiss “DRC-Group” company) modified by an electromagnetic field. As a result of the experimental part of the study, certain changes in the properties of the R.O.C.S. Active Calcium toothpastes under the electromagnetic field influence including microstructural transformation of the toothpaste (an increase in the size of the polymer matrix particles and a decrease in the distance between them confirmed by the electron microscopy findings), confirmed by the transmission electron microscopy results. The investigations with the help of X-ray microanalysis made it possible to clarify the chemical composition of the surface layer of the tooth enamel, for the first time to reveal in detail the concentration of micro- and macroelements of organic and inorganic nature, to trace the changes occurring under controlled tooth cleaning with the R.O.C.S. Active Calcium toothpaste treated with an electromagnetic field. The obtained results promote the further studies of laws in change of physical and mechanical properties of treatment-and-prophylactic means under the electromagnetic field influence in respect of dental caries preventive maintenance efficiency increase.

Keywords Individualized prevention of initial dental caries · Microstructural transformation of the polymer-based toothpastes · Electromagnetic field · Transmission electron microscopy (TEM) · Scanning electron microscopy (SEM) · X-ray spectral microanalysis

Background In the twenty-first century, tooth decay remains the main problem of dentistry due to the high prevalence among the world population according to the World Health Organization [1–3]. In connection with this, the most

important tasks of modern dentistry cover not only early diagnosis but also prevention of dental caries including the one based on the use of various hygienic products and therapeutic agents [1, 4].

Nowadays, more and more therapies are used both to prevent the development of caries and to treat its initial stage. However, in a number of cases, we do not know the delicate mechanisms of these processes. All this indicates the necessity and importance of studying the chemical composition of tooth tissues, especially, enamel with the aim of effective control over the maturation and remineralization processes that make dental caries prevention and treatment effective [4–7].

Normally, the processes of re- and demineralization in the oral cavity are in a state of dynamic equilibrium. However, in the presence of cariogenic factors, the balance is shifted towards demineralization. Enamel resistance to dental caries

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depends on internal and external factors, but one of the leading one is dental plaque. The accumulation of positive (mineral) components from toothpastes in dental plaque is the main factor providing enamel exchange processes; therefore, dental enamel permeability as a physiological indicator of resistance is important in solving the problem of dental caries [8, 9]. The study of tooth enamel permeability at early stages of caries development requires particular attention [10, 11].

One of modern directions of research dedicated to dental caries prevention is based on a well-known phenomenon of remineralization of hard tooth tissues by compensating enamel mineral components. Remineralization is based on the adsorption of minerals into carious areas [4]. A criterion for the effectiveness of remineralizing solutions is the disappearance or reduction of carious spots, a decrease in the growth of dental caries. The possibility of active impact of remineralizing preparations on the degree of mineralization of enamel is now experimentally and theoretically proved [10, 12].

The mechanism of demineralization and remineralization of enamel lies in the fact that they remain in a dynamic balance, in a “mouth fluid-enamel” system. This causes an essential process of renewal of substances which occurs in hard tooth tissues in norm [8, 10]. The shift of the balance towards demineralization marks the beginning of pathological structural changes in tooth enamel [4, 6]. In the event of initial caries, enamel remineralization is possible only to a certain extent and, specifically, for a reversible degree of carious lesion.

At the stage of initial caries, with a partial loss of mineral substances (demineralization), free microspaces are formed but a protein matrix capable of remineralization is preserved. The increased permeability of enamel causes the penetration of calcium and phosphates ions from saliva or artificial remineralizing solutions into the demineralization area followed by the formation of hydroxyapatite crystals and the filling of enamel microspaces [4].

If tooth enamel is saturated with organic and mineral elements (calcium, phosphorus, fluorine, magnesium) in time, it is possible to achieve its complete restoration and to increase its resistance to dental caries [5, 8], which should be promoted by therapeutic and preventive toothpastes.

With the purpose of remineralization of hard tooth tissues, many researchers [8, 10, 12] used a wide range of remineralizing agents containing necessary mineral components such as Ca, P, F, and Mg, taking into account their compatibility and the caries static effect strength.

For a long time, the inclusion of fluoride in a toothpaste was considered an effective method of dental caries prevention [8]. The use of fluoride preparations is based on a variety of fundamental and applied research in different countries [10–12]. The effect was especially evident in children [9].

However, in the 2000s, the studies were conducted to ascertain that fluorine is not embedded in the crystal lattice after 15 years of age (our works).

The results of a study of morphology and ultrastructure as well as the microchemical component of hard tooth tissues known since 1953 [4, 6] made it possible to detect the positive effect of various toothpaste constituents on metabolic processes in hard tooth tissues and, especially, in enamel.

A.A. Kunin's 1-month studies of tooth enamel (2004) performed with the help of scanning electron microscopy (SEM) and X-ray microchemical analysis (PMA) using various toothpastes showed the greatest effect of fluoride-containing Colgate toothpastes and, first of all, ROCS in 85% of children up to 15 years. The same author studied the content of microelements (Ca, Mg, S, F, P, Si, etc.) of tooth enamel in patients of older age groups (from 20 to 70 years old) and proved that fluorine practically does not improve metabolic processes in hard tooth tissues in these age groups and that magnesium is more important. Those results were the reason for this study [4, 8].

Presently, the use of toothpastes containing magnesium is topical. The effectiveness of magnesium has been proved in scientific studies conducted by the Therapeutic Dentistry Department of VSMU named after N.N. Burdenko [8].

A variety of caries prevention drugs currently complicates their choice by dentists. If you rely on methods of mass prevention, then, according to many data, the effect will be far from optimal but in certain social groups of the population it still creates the probability of a positive trend. From these perspectives, it is necessary to take into account the results of scientific research indicating various options for activating metabolic processes in hard tooth tissues. Thus, for example, the studies of metabolic processes in enamel and dentin of beavers' teeth showed an increased concentration of fluoride in temporary teeth of baby beavers and a corresponding tendency for magnesium in permanent teeth of adult beavers [4]. Other researchers stated that, with the increased content of magnesium in toothpastes, the level of metabolic processes in hard tooth tissues improves, which increases their resistance to caries [8]. This pattern was revealed in the study of enamel metabolism with due consideration of its microstructure and the movement of organic and inorganic substances through enamel tunnels [13].

It is known that therapeutic and prophylactic toothpastes for prevention of dental caries consist of a number of components including polymers, fine inorganic filler (silicon, magnesium, calcium glycerophosphate, titanium dioxide, etc.) as well as antibacterial and foam-forming components. All components take part in the formation of the basic structure of these toothpastes and characterize their therapeutic and chemical properties.

There was an idea about structural changes in polymer-based consumable dental materials including toothpastes after the electromagnetic exposure [8, 14, 15]. The obtained effect resulted in an increase in the size of particles of the polymer base. Such a reaction can be predicted not only in toothpastes; similar effects were obtained in the study of polymer restorative materials for treatment of dental caries.

But the same polymers are found in toothpastes as well. Our long-term studies of toothpastes (especially DRC-group) showed a pronounced activity of their components; therefore we took one of the pastes for the study.

On the way to creating an “ideal” toothpaste, new technologies are being introduced that improve their physical-chemical and physical-mechanical characteristics. Special attention in improving physical-chemical characteristics of polymer-based toothpastes is given to methods of intended change of morphology of polymer-based materials by affecting them by thermal and chemical treatment, irradiation, and also by magnetic, electric, or ultrasonic fields.

The studies carried out by national and foreign scientists to detect the magnetic and electric fields impacts on the structure change in liquid polymers have shown the improvement in their physical-mechanical characteristics [3, 16, 17].

The purpose of the study is to increase the efficiency of dental caries prevention based on the activation of metabolic processes in hard dental tissues with the use of R.O.C.S. Active Calcium toothpastes on a polymer base modified by electromagnetic field.

Materials and methods

The study was performed at the Department of Hospital Dentistry and Oral and Maxillofacial Surgery of Voronezh State Medical University named after N.N. Burdenko in co-operation with Voronezh State University (the Center for collective use of scientific equipment), the Department of Electrical, Heat and Hydraulic Engineering of Voronezh State Forestry University named after G.F. Morozov and Periodontology Department of Cukurova University.

The study was approved by the local committee for ethics in research of State Funded Educational Institution of Higher Education “Voronezh N.N. Burdenko State Medical University” of the Ministry of Health of the Russian Federation (Protocol No. 1, February 25, 2016) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The written informed consent was obtained from all the patients. The participants were randomly selected from a pool of patients (18–35 years old) who had sought dental treatment.

The material obtained in the experimental and clinical study was analyzed in a series of clinical-anamnestic and clinical-laboratory tests.

To carry out the experimental part of the research, we studied the polymer-based R.O.C.S. Active Calcium toothpaste produced by the Russian-Swiss “DRC-Group” company (“R.O.C.S.” (remineralizing oral care systems) is an abbreviation of the toothpastes and means of oral hygiene. The products of the R.O.C.S. brand are manufactured by DRC-Group, Russian-Switzerland Company. The name of commodity in our research study is R.O.C.S. Active Calcium toothpaste) containing such mineral components as calcium, phosphorus, silicon, and magnesium in the optimal ratio for teeth, as well as xylitol (6%)—an antibacterial component of plant origin that prevents the development of cariogenic microflora and the formation of plaque. When applied to the tooth surface, the toothpaste forms a film and ensures a gradual penetration of the active components into the surface layers of tooth enamel.

The application of the R.O.C.S. Active Calcium toothpaste was supported by patients’ interviewing which showed the high prevalence of its use among the population and the fact that this toothpaste is sold in different countries. In addition, it contains active organic polymers which are more important for the reaction to electromagnetic field.

The basis for obtaining scientific data, according to the purpose and objectives, was a group of 62 people aged from 18 to 35 (i.e., with the completed mineralization of hard tooth tissues) without pronounced concomitant pathologies who had orthodontic, surgical, or periodontal indications for the removal of intact teeth. The surveyed contingent was divided into two equivalent groups consisting of 31 people (I—the study group of patients using toothpaste R.O.C.S. Active Calcium with the electromagnetic field influence; II—control group using toothpaste R.O.C.S. Active Calcium without the electromagnetic field influence). The laboratory research included the study of microstructural features of polymer-based toothpastes under the influence of electromagnetic field and without it as well as morphochemical studies involving the study of extracted teeth.

Methods of the study

To implement the study of the electromagnetic field influence on the microstructure and the physical-chemical properties of toothpastes containing polymers, a special device for polymeric materials processing in a constant electromagnetic field (EMF) was used [5, 15]. The basis of this installation is an electromagnetic inductor consisting of two movable coils of an electromagnet connected in series on a magnetic yoke. Magnetizing coils made of wire of the PSD brand are interconnected in series. The electromagnet is connected to a special power unit, from which a current of up to 12 A is applied to the winding of the coils.

The activation of a polymer by magnetic field in a fluid-like state leads to a change in its physical-mechanical characteristics. The change in the properties of the polymer is a reflection of the processes occurring in the polymer structure. The changes that occur in the polymer structure under the action of magnetic field are caused by the anisotropy of the diamagnetic susceptibility of macromolecules and their parts [5, 15].

The toothpaste processing in the device was carried out in the following order. The toothpaste in the factory packaging was placed in the working inter-pole space for electromagnetic processing of polymeric materials in a constant electromagnetic field. The experiment was performed at a given magnetic field strength of 20×10^4 – 24×10^4 A/m for 20 min.

The microstructure and the size of the toothpaste material particles were examined by transmission electron microscopy (TEM) using a Libra 120 microscope (Carl Zeiss, Germany) with an integrated OMEGA filter for studying polymer samples and a Koehler illumination system in the mode of elastically scattered electrons with an accelerating voltage of 120 kV. The technique [17] of analysis of the structure in the light-field mode of the microscope operation was applied.

The samples for TEM were prepared by immersing an amorphous carbon film (10 nm thick) fixed in a slide grid into an aqueous solution of a toothpaste dispersed by ultrasound. The sonication was performed on an ultrasonic disperser (homogenizer) Sonicator Q5003 (QSONICA, USA). Excessive moisture from the sample was removed on an impregnated filter. The average particle size of the toothpaste samples was determined according to the Jeffrey's Method [7]. Statistical processing was carried out with the Altami Studio program (version 3.4; <http://altamisoft.ru>; Altami LLC).

In the same manner, the R.O.C.S. Active Calcium toothpaste not subjected to preliminary treatment by electromagnetic field (EMF) was examined.

The experimental studies made it possible to determine the prerequisites for clinical study of the effects of the R.O.C.S. Active Calcium toothpaste exposed to electromagnetic field on the surface layer of tooth enamel.

Clinical research methods allowed forming groups of patients to determine indications for further laboratory studies and to justify indications for tooth extraction.

The functional state of enamel was determined using the enamel resistance (TER) test according to V.R. Okushko, L.I. Kosareva, 1983. A drop of 1 N hydrochloric acid solution was applied to the previously cleaned vestibular surface of the central upper incisor. After 5 s, it was washed with water; the tooth was dried; a drop of 2% methylene blue solution was applied to the etching site. The degree of coloration was evaluated according to a standard 10-point blue scale. If the intensity of coloring of the etched area of enamel

corresponded to a color intensity of 10–30%, a high resistance of the teeth to caries was predicted; 40–60% meant a medium degree while 70% and more meant the low one.

The obtained results made it possible to assess the tooth enamel susceptibility and resistance to dental caries in the examined patients and to detect the early signs of its initial stage.

The assessment of dental plaque cariogenicity before and after controlled tooth cleaning was carried out according to the procedure proposed by J.L. Hardwick, E.B. Manly, 1952; the acid activity was determined with the use of the colorimetric method of indicator's color changing from yellow to pale orange (0–30%); from pale orange to red (40–60%); from red to dark red (70–100%); methylene red was used as a color indicator for dental plaque. One percent of glucose solution was applied for several minutes on the tooth enamel; 0.1% aqueous solution of methylene red was applied on the surface of lower incisors. The results of staining were interpreted in the following way: the indicator was positive if the color of the stained plaque changed to red; the indicator was negative if the color did not change.

Subsequently, the examined contingent of 62 patients was divided into two equivalent groups of 31 people.

The patients from the both groups underwent controlled tooth cleaning using the R.O.C.S. Active Calcium toothpaste daily during a month. In group I, the patients were prescribed controlled tooth cleaning using the R.O.C.S. Active Calcium toothpaste (DRC-Group, Russia-Switzerland) pretreated in an electromagnetic field. The patients from group II were control subjects who used the R.O.C.S. Active Calcium toothpaste not subjected to preliminary treatment by an electromagnetic field.

To analyze the permeability of tooth enamel for toothpaste chemical components with and without the electromagnetic field influence in vitro, 12 intact teeth removed due to orthodontic, surgical and periodontal indications were assessed using quantitative X-ray spectrum microchemical analysis (XRSA) and, according to the study groups, were subjected to the controlled cleaning with the R.O.C.S. Active Calcium toothpaste during one month.

The preparation of tooth chippings for examination was as follows: the removed intact teeth were washed with running water, dried with filter paper, and then a notch (approximately 3 mm deep) was placed with a diamond disc. Using a dental carver, the sample was cut along the marked notch so that the tooth was chipped and the area of the viewed surface was not less than 0.5 cm.

The prepared chippings of the removed teeth were fixed on a holder, placed in the JEOL “JSM-638OLV” (Japan) scanning electron microscope chamber and subjected to a focused electron beam going into raster on the sample surface. The images were obtained in the secondary-electron emission regimes in a low-vacuum SEM. The research analysis of X-ray

radiation gave qualitative and quantitative data on the elemental composition of the object under study.

The distribution of chemical elements in the surface layer of enamel was studied using XRSA with mapping of lateral chippings with the INCA-250 energy-dispersive analysis system. The distribution of chemical elements on the X-ray map was marked with color (red, green, blue) and characterized the level of toothpaste components penetration into the tooth enamel.

Statistical analyses

For statistical processing of the results of the study, a standard package of STATISTICA 8.0 software programs from Statsoft was used [18]. Before the statistical analysis of the results of the study, a concrete copy of the program was verified on test samples with known properties and results. Within the framework of descriptive (discriminant) statistics, the following parameters were calculated: mean and standard deviation, median and standard error of the excess, kurtosis, asymmetry, minimum and maximum value of the data series, quantile value, quantile range. To compare the groups, we used a

nonparametric criterion for the difference between Friedman Anova, Kruskal-Wallis, and the median test, taking into account the effect of multiple comparisons. When processing statistical data, differences were considered significant at $p < 0.05$.

Results and discussion

Using a LIBRA 120 PLUS (Zeiss) transmission electron microscope with a built-in OMEGA filter for studying polymer samples and the Koehler illumination system, the microstructural features of the R.O.C.S. Active Calcium toothpaste influenced and not influenced by an electromagnetic field were studied.

The results of TEM confirm certain changes in the toothpaste microstructure under the electromagnetic field influence: the toothpaste polymer matrix particles densification and enlargement (Fig. 1a–f, Table 1).

Figure 1a–f shows that under the electromagnetic field influence, the toothpaste sample has a more ordered structure; it is more dispersed and has microstructural changes in comparison with the toothpaste without such an influence. The

Fig. 1 The light-field TEM images of the toothpaste R.O.C.S. specimens **a, d** before the electromagnetic field exposure, **b, e** after the electromagnetic field, and **c, f** 3 months after the electromagnetic field exposure

Table 1 Comparative characteristics of microstructure of toothpaste R.O.C.S. on a polymer basis from transmission electron microscopy (TEM)

Toothpaste R.O.C.S. Active Calcium	Meaning	Particle size ($n = 15$)			Distance between the particles ($n = 15$)		
		Before	After	After 3 months	Before	After	After 3 months
500 nm	$M \pm m$	$36.49 \pm 1.99^*$	$41.27 \pm 3.17^*$	40.01 ± 3.39	$56.41 \pm 0.29^*$	$34.0 \pm 2.68^*$	35.26 ± 2.75
200 nm		$41.95 \pm 2.58^*$	$46.32 \pm 3.53^*$	46.31 ± 3.51	$50.33 \pm 0.33^*$	$40.13 \pm 3.03^*$	40.39 ± 3.1

$p < 0.05$

structure of the toothpaste subjected to an electromagnetic field becomes denser due to the reduced distance between the particles and the enlargement of their size; there is a distinct difference in comparison with the control group. The toothpaste from the control group has large gaps between the particles while the structure of the toothpaste exposed to an electromagnetic field is thicker and denser.

To confirm the obtained results, we analyzed the changes occurring in the toothpaste structure after the electromagnetic field influence including the determination of the particle size and the distance between them. The data are presented in Table 1 and Fig. 1a–f.

The data of Table 1 and Fig. 1a–f representing the microstructure of the R.O.C.S. toothpaste, DRC-Group, Russia-Switzerland before and after the exposure to an electromagnetic field indicate the enlargement of the particles and the reduction in the distance between them in the toothpaste of the study group influenced by an electromagnetic field compared with the control group toothpaste without such an influence. The differences between the study groups based on this characteristic are statistically significant ($p < 0.05$).

The changes in the microstructure of the R.O.C.S. Active Calcium toothpaste on a polymer basis, DRC-Group, Russia-Switzerland ($\times 500$ nm) before and after the exposure to an electromagnetic field are the following: in the control group, the average size of the toothpaste particles was 36.49 ± 1.99 μm ; in the study group, it was 41.27 ± 3.17 μm ; 3 months later, it was 40.01 ± 3.39 μm , which indicates the particles enlargement in comparison with the control group. The distance between the toothpaste particles before the exposure was 56.41 ± 0.29 μm ; after the exposure, it was 34.0 ± 2.68 μm in different parts of the visual field; 3 months later, it was 35.26 ± 2.75 μm , which indicates the densification of the toothpaste microstructure. The differences between the study and control groups based on this characteristic are statistically significant ($p < 0.05$) (Table 1, Fig. 1a–c).

When studying the R.O.C.S. Active Calcium toothpaste, DRC-Group, Russia-Switzerland ($\times 200$ nm), the enlargement of the particles and the densification of the toothpaste microstructure after the electromagnetic field action were also observed. Thus, in the control group, the average size of the particles was 41.95 ± 2.58 μm ; in the study group, it was

46.32 ± 3.53 μm ; 3 months later, it was 46.31 ± 3.51 μm . The distance between the toothpaste particles before the exposure was 40.13 ± 3.03 μm ; after the exposure, it was 40.13 ± 3.03 μm ; 3 months later, it was 40.39 ± 3.1 μm . The differences between the study and control groups based on this characteristic are statistically significant ($p < 0.05$) (Table 1, Fig. 1d–f).

Thus, the achieved effect of the particles enlargement and the densification of the microstructure of the R.O.C.S. Active Calcium toothpaste after the exposure to an electromagnetic field remained unchanged after 3 months of the follow-up period.

The clinical studies included the TER test in patients at the initial stage of the examination, which contributed to the identification of a caries-susceptible group before the use of targeted controlled tooth cleaning. In the course of the study, a definite relationship between the TER test findings and the plaque cariogenicity degree was revealed. The table presents the examined patients' TER test results, making it possible to predict the tooth enamel resistance in patients (Table 2).

According to the TER test, a moderate tooth enamel resistance to the acid buffer was mostly determined before controlled tooth cleaning and it was increased after it in the both groups. However, the effect in the study group using the R.O.C.S. Active Calcium toothpaste exposed to an electromagnetic field was more pronounced. In the second group using the R.O.C.S. Active Calcium toothpaste without such an influence, there were no significant changes (Table 2).

The study resulted in the fact that the majority of patients from the both groups had a medium level of dental plaque cariogenicity before controlled tooth cleaning and its decrease after the procedure. However, the effect in the study group using the R.O.C.S. Active Calcium toothpaste exposed to an electromagnetic field was more pronounced, which indicates the beneficial effect of therapeutic physical factors in terms of eliminating dental plaque cariogenicity. In the second group using the R.O.C.S. Active Calcium toothpaste without the electromagnetic field influence, the changes were not significantly pronounced (Table 2).

To study the relief of the intact tooth enamel surface layer after controlled cleaning with the toothpaste with and without the electromagnetic field action, SEM was performed. In the

Table 2 The distribution of patients according to the data of the test of enamel resistance (TER test) and plaque cariogenicity before and after controlled tooth cleaning

Type of research	Clinical groups	Total amount of people		
		Before tooth brushing (control group)	After tooth brushing	
			Group I with EP	Group II without EP
The level of resistance according to the TER test results	High (0–30%)	10 (16.13%)	9 (14.52%)	6 (9.68%)
	Middle (30–60%)	35 (56.45%)	18 (29.03%)	17 (27.42%)
	Lower (60–100%)	17 (27.42%)	4 (6.45%)	8 (12.9%)
	Total	62 (100%)	31 (50%)	31 (50%)
The level of the tooth plaque cariogenicity	Lower (0–30%)	8 (12.9%)	11 (17.74%)	5 (8.06%)
	Middle (40–60%)	36 (58.06%)	15 (24.18%)	17 (27.42%)
	High (70–100%)	18 (29.04%)	5 (8.06%)	9 (14.52%)
	Total	62 (100%)	31 (50%)	31 (50%)

electronic image (Fig. 2), there were well-marked dental tubules lines in the form of tunnels penetrating into the enamel deep layers and surface evenly coated with the particles of the toothpaste treated with an electromagnetic field.

To assess the microchemical effect of the toothpaste, the enamel surface layer was scanned using the targeted X-ray spectrum microchemical analysis. In the study of the energy spectrum, we quantified the microelement composition of chemical elements on the tooth enamel surface of the study groups subjected to controlled cleaning using the R.O.C.S. Active Calcium toothpaste with and without the electromagnetic field action (Fig. 3a, b, Tables 3, 4).

The scanning of the enamel surface layer after controlled tooth brushing with the toothpaste exposed to an

electromagnetic field revealed a higher percentage of microelements characterizing the penetration of mineral components of the therapeutic R.O.C.S. Active Calcium toothpaste into the intercrystalline spaces of the enamel surface layer: an increase in the weight percentage of calcium, 18.27 ± 0.56 ; phosphorus, 10.58 ± 0.25 ; carbon, 29.35 ± 0.42 ; and magnesium, 0.7 ± 0.02 as the main components of the toothpaste.

The enamel surface layer turned out to be more saturated with mineral elements than the enamel layer without the electromagnetic field influence (Table 4).

Any fundamental research should be based on comprehensive clinical studies (TER test and dental plaque cariogenicity), which allows evaluating the simplest variants of metabolic processes in hard dental tissues and planning more extensive research.

SEM and X-ray spectrum microchemical analysis (XRSA) of tooth enamel make it possible to estimate the level of hygienic and preventive remedies influence on the metabolic processes in enamel in the context of increasing its resistance to cariogenic factors.

Conclusions

The study analyzed the structural changes in consumable dental materials on a polymer basis including toothpastes. The obtained effect resulted in size increasing of the polymer base particles. Such a reaction can be predicted not only in toothpastes; similar effects were obtained in the study of polymer-based restorative materials for dental caries treatment.

But the same polymers are found in toothpastes. Our long-term studies of toothpastes (especially ones by DRC-Group)

Fig. 2 Dental tubules lines on the enamel surface coated with the toothpaste particles. The electronic image of the tooth chipping in the regime of secondary electron emission at $\times 500$ magnification

Fig. 3 X-ray spectrum microanalysis of the distribution of chemical elements of the enamel surface. The electronic image of the intact tooth enamel surface after controlled tooth cleaning with the toothpaste treated with an electromagnetic field. The scanning zone in the enamel surface layer at $\times 500$ magnification

showed a pronounced activity of their components [4, 5]; that was the reason why we took one of them for our study.

Scanning electron microscopy and X-ray spectrum analysis of tooth enamel make it possible to estimate the level of hygienic and preventive remedies influence on the metabolic processes in enamel in the context of increasing its resistance to cariogenic factors. The analysis of the obtained data indicates the presence of changes occurring in the microstructural features of the toothpaste and physical-chemical properties of enamel under the influence of the therapeutic R.O.C.S. Active Calcium toothpaste treated with an electromagnetic field.

The result of the experimental part of the study was that we revealed certain changes in the properties of the R.O.C.S. Active Calcium toothpaste exposed to an electromagnetic field including microstructural transformations of the toothpaste: an increase in the size of the polymer matrix particles and a decrease in the distance between them confirmed by the electron microscopy findings and statistics.

Also, we have carried out a program calculation of electronic images to confirm the general tendency of particle enlargement by the Jeffries method [7]. According to the received results from electronic images, the tendency of

particles enlargement after the electromagnetic exposure is maintained, as in the case of nonautomated counting. In Fig. 4a–f, there are the distribution curves of the light-field TEM images of toothpaste specimens. For samples a and d, the average particle size was 15 nm, and for samples b and e, it was 20 nm. As for the c and f images, there were two-mode distribution of particle sizes with a medium sizes predominance – 20 and 80 nm. The differences between the study groups based on this characteristic are statistically significant ($p < 0.05$) (Fig. 4a–f).

X-ray spectrum microanalysis made it possible to clarify the chemical composition of the tooth enamel surface layer, to reveal in details the concentration of micro- and macroelements of organic and inorganic nature, to trace the changes occurring in the course of controlled tooth cleaning with the R.O.C.S. Active Calcium toothpaste treated with an electromagnetic field.

When examining the samples of 12 tooth chippings by X-ray spectrum microanalysis and mapping following the preliminary exposure of the toothpaste to an electromagnetic field, an increase in the concentration of calcium, phosphorus, magnesium, and oxygen in the

Table 3 Quantitative elemental chemical composition of surface enamel layer of intact tooth with and without the EP exposure

Element	With the EP exposure (a)		Without the EP exposure (b)	
	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)
C	28.72	41.41	18.42	27.52
O	39.86	43.15	50.76	56.92
Na	0.64	0.48	0.70	0.55
Mg	0.75	0.53	0.25	0.18
P	11.38	6.36	11.06	6.40
Ca	18.65	8.06	18.59	8.32
Cl			0.22	0.11
Total	100.00		100.00	

Table 4 Distribution of micro- and macroelements in the surface layer of the enamel of intact teeth with and without the EP exposure (weight%, $M \pm m$)

Element	Weight coefficient, %	
	Surface layer of enamel with the EP exposure	Surface layer of enamel without the EP exposure
C	$29.35 \pm 0.42^*$	$19.63 \pm 0.2^*$
O	42.2 ± 0.46	42.23 ± 0.41
Na	0.66 ± 0.01	0.62 ± 0.02
Mg	$0.7 \pm 0.02^*$	$0.3 \pm 0.01^*$
P	$10.58 \pm 0.25^*$	$9.59 \pm 0.28^*$
Cl	$0.11 \pm 0.01^*$	$0.26 \pm 0.01^*$
Ca	$18.27 \pm 0.56^*$	$16.42 \pm 0.29^*$

* $p < 0.05$

tooth enamel surface layer was recorded in the electronic image (Fig. 3a, Table 3) in the secondary emission mode, which was confirmed by the penetration and accumulation of the components of the R.O.C.S. Active Calcium toothpaste treated with an electromagnetic field in the intercrystalline spaces of the enamel surface according to the results of planar graphs coloring. Magnesium as a characteristic component of the

toothpaste is visually accumulated in the enamel surface layer (Fig. 5c, Table 4).

The received results of the research have shown certain laws in change of physical and mechanical properties of toothpastes on a polymeric basis under the electromagnetic field influence. According to the TER test, there was an increase in the tooth enamel resistance and a reduction in plaque cariogenicity in the

Fig. 4 The distribution curves of particles enlargement with and without the electromagnetic exposure. **a, d** Before the electromagnetic exposure. **b, e** After the electromagnetic exposure. **c, f** Three months after the electromagnetic exposure

Fig. 5 X-ray map of the planar distribution of chemical elements on the enamel surface. **a, b** C, red; Ca, green; P, blue; **c, d** Mg, red; Na, green; O, blue. Energy-dispersive X-ray analysis of scanning electron microscopy at $\times 500$ magnification

study group of patients using toothpaste treated with an electromagnetic field. The results of the conducted studies can find their application in terms of increasing caries prevention.

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Authors' contributions NM substantially contributed to the scientific conception and design, acquisition of the data, and analysis and interpretation of the data, carried out the scientific studies, participated in the sequence alignment, conceived the study, participated in its design and coordination, and drafted the manuscript. AK participated in its design and coordination and drafted the manuscript. CH has contributed to the data collection and concepts' development. All authors read and approved the final manuscript.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Consent for publication Not applicable.

Ethical approval All the patients were informed about the purposes of the study and consequently have signed their "consent of the patient." All investigations conformed to the principles outlined in the Declaration of Helsinki and were performed with permission by the responsible Ethics Committee of State Funded Educational Institution of Higher Education "Voronezh N.N. Burdenko State Medical University" of the Ministry of Health of the Russian Federation (Protocol No. 1, February 25, 2016).

References

1. Golubnitschaja O, Watson ID, Topic E, Sandberg S, Ferrari M, Costigliola V. Position paper of the EPMA and EFLM: a global vision of the consolidated promotion of an integrative medical approach to advance health care. *EPMA J.* 2013;4(1):12. <https://doi.org/10.1186/1878-5085-4-12>.
2. Kunin AA, Belenova IA, Ippolitov YA, Moiseeva NS, Kunin DA. Predictive research methods of enamel and dentine for initial caries detection. *EPMA J.* 2013;4(Suppl 1):19. URL: <http://www.epmajournal.com/content/4/1/19>.
3. Golubnitschaja O, Baban B, Boniolo G, Wang W, Bubnov R, Kapalla M, et al. Medicine in the early twenty-first century: paradigm and anticipation—EPMA position paper 2016. *EPMA J.* 2016;7:23. <https://doi.org/10.1186/s13167-016-0072-4>.
4. Moiseeva NS, Kunin AA, Kunin DA. The new direction in caries prevention based on the ultrastructure of dental hard tissues and filling materials. *EPMA J.* 2016;7(Suppl 1):A49. <https://doi.org/10.1186/s13167-016-0054-6>.
5. Kunin AA, Moiseeva NS, Mekhantieva LE. Improving the effectiveness of dental caries prevention using therapeutic toothpastes. *EPMA J.* 2017;8(Suppl 1):S50. <https://doi.org/10.1007/s13167-017-0108-4>.
6. Dyonosopulos R, Gerasimou P, Tolidis K. The effect of home-use fluoride gels on glassionomer, compomer and composite resin restorations. *Oral Rehabil.* 2003;30(7):683–9.
7. Maunsbach AB, Afzelius BA. Biomedical electron microscopy—illustrated methods and interpretations. London: Academic Press; 1999. 548 p.
8. Artelt HM. Fluoridpräparate bei der Behandlung überempfindlicher Zahnhalse. *Swiss Dent.* 1994;15(4):9–13.
9. Dykstra MJ, Reuss LE. Biological electron microscopy. theory, techniques, and troubleshooting, vol. 360. New York: Plenum Press; 2003. p. 9. <https://doi.org/10.1002/sca.4950150411>.
10. Arnold W. Volumetric assesment and quantitative element analysis off the effect of fluoridated milk on enamel deminicalisation. *Arch Oral Biol.* 2003;48:467–73. [https://doi.org/10.1016/S0003-9969\(03\)00087-6](https://doi.org/10.1016/S0003-9969(03)00087-6).

11. Kryzhanovskij VK, Burlov VV, Panimatchenko AD, Kryzhanovskaya YUV (2005) *Tekhnicheskie svoystva polimernykh materialov: Ucheb.-sprav. posobie. 2-e izd., ispr. i dop.* – SPb.: Professiya. 248 s.
12. Moiseeva NS, Kunin AA (2016) Method of improving the adhesion and strength properties of dental filling materials and bonds. RF patent 2594255.
13. Cutress T. Caries preventive effect of high fluoride and xylitol containing dentifrices. *J Dent Child.* 1992;59:313–8.
14. Zolotova LYU, Korshunov AP. Vliyanie glubokogo fluorirovaniya na dinamiku mineralizatsii dentina u pacientov s razlichnymi urovnymi rezistentnosti k karies. *Institut Stomatologii.* 2003;3: S56–7.
15. Moiseeva NS, Kunin AA, Shabanov RA. Electromagnetic influence on microstructural changes in dental filling materials: improvement in physical and mechanical properties. *EPMA J.* 2017;8(Suppl 1):S49. <https://doi.org/10.1007/s13167-017-0108-4>.
16. Popov VM, Latynin AV (2014) K sozdaniyu kleenoi drevesiny povyshennoy prochnosti na osnove kleev, modifitsirovannykh vozdeystviem kombinirovannykh fizicheskikh polej. Aktual'nye napravleniya nauchnykh issledovaniy XXI veka: teoriya i praktika–T.2, no. 2.1. *Rezhim dostupa.* <https://doi.org/10.12737/3004>.
17. Dogan MS, Yavas MC, Yavuz Y, Erdogan S, Yener İ, Simsek İ, et al. Effect of electromagnetic fields and antioxidants on the trace element content of rat teeth. *Drug Des Devel Ther.* 2017;11: 1393–8. <https://doi.org/10.2147/DDDT.S132308>.
18. Glanc S. Mediko-biologicheskaya statistika. per. s angl. – M, Praktika, 459 s. 1998. <http://www.medstatistic.ru/articles/glantz.pdf>.