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The Evaluation of Efficiency of the Impact of Vitamin D and Remineralizing Toothpaste on the Structure of the Dental Enamel in the Individuals with Homozygous Polymorphism in the Gene, Encoding the Intracellular Vitamin D Receptor (VDR)

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ABSTRACT

BACKGROUND: *Only a few literature sources data show the relation of the VDR gene polymorphism and the susceptibility to developing dental caries. Within this context, investigating the structure of the dental enamel and the changes of its resistance under the effects of vitamin D and remineralizing therapy among the persons with the homozygous polymorphism of the VDR gene (A/A) is topical.* ***AIM:*** *to investigate the effects of vitamin D and the toothpaste with remineralizing contents on the structure of the enamel surface of the impacted teeth extracted from the individuals with homozygous polymorphism of the VDR gene.* ***METHODS:*** *In 2023–2025, within the premises of the Dentistry Department of the Federal State Budgetary Institution of Continuing Professional Education "Central State Medical Academy", a total of 200 students aged 22–25 years were screened with undergoing a genetic testing to reveal the polymorphism of the VDR gene. Out of the 36 assessed subjects, 18 individuals were detected with the homozygous A/A allele that are currently undergoing orthodontic therapy and requiring an extraction of the impacted molars. A total 24 of extracted teeth were tested with submerging them into the artificial saliva with an addition of various media. The dental samples were distributed into four groups: only artificial saliva (I, control); 1000 IU of cholecalciferol per 100 ml (II); processing with remineralizing toothpaste (III); vitamin D and remineralizing toothpaste (IV). The evaluation of the structure of the dental enamel was carried out using the method of confocal profilometry with measuring the Ra and Rp roughness parameters.* ***RESULTS:*** *In group II with the presence of cholecalciferol, changes were revealed in the roughness parameters (Ra, Rp) of dental enamel surface, in group III (processing with remineralizing toothpaste) the Ra and Rp parameters had similar digital values. As for the samples from the group IV, comparing to the group I, smoothness was revealed in the dental enamel surface, which is confirmed by the Ra and Rp ($p > 0.001$) parameters. This effect can be explained by the synergic action of the cholecalciferol and the remineralizing components of the toothpaste on the structure of the enamel.* ***CONCLUSION:*** *In the individuals with homozygous polymorphism (A/A) of the VDR gene, significant changes were revealed in the parameters of dental enamel roughness (Ra and Rp) after the combined use of cholecalciferol and remineralizing toothpaste, which is related to the smoothening of surface due to the formation of the homogeneous layer consisting of the microRepair microcrystals.*

Keywords: *polymorphism; VDR gene; confocal profilometry; remineralizing toothpaste; vitamin D; cholecalciferol.*

For citation:

Tikhonova SN, Kozlova MV, Gorbatova EA. The Evaluation of Efficiency of the Impact of Vitamin D and Remineralizing Toothpaste on the Structure of the Dental Enamel in the Individuals with Homozygous Polymorphism in the Gene, Encoding the Intracellular Vitamin D Receptor (VDR). *Journal of Clinical Practice*. 2025;16(2):41–52. doi: 10.17816/clinpract678365 EDN: MJYCOQ

Submitted 10.04.2025 Accepted 13.06.2025 Published online 27.06.2025

BACKGROUND

According to the results of researches on dental health, the incidence and the intensity of dental caries remains high despite the conducted prevention measures and modern treatment methods [1].

The susceptibility of dental enamel to the effects of cariogenic factors is mainly determined by its

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Оценка эффективности действия витамина D и реминерализирующей пасты на структуру эмали зубов у лиц с гомозиготным полиморфизмом гена, кодирующего внутриклеточный рецептор витамина D

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АННОТАЦИЯ

Обоснование. *Единичные литературные данные указывают на связь полиморфизма гена внутриклеточного рецептора витамина D (VDR) с предрасположенностью к развитию кариеса. В связи с этим изучение структуры эмали зубов и изменений её резистентности под действием витамина D и реминерализирующей терапии у лиц с гомозиготным полиморфизмом гена VDR (A/A) является актуальным.* ***Цель исследования*** *— изучить действие витамина D и зубной пасты с реминерализирующим составом на структуру поверхности эмали удалённых ретенированных зубов у лиц с гомозиготным полиморфизмом гена VDR.* ***Методы.*** *В 2023–2025 годах на кафедре стоматологии ФГБУ ДПО ЦГМА обследовано 200 обучающихся в возрасте 22–25 лет, которым проведён генетический тест на определение полиморфизма гена VDR. Выявлено 18 человек с гомозиготным аллелем A/A, находившихся на ортодонтическом лечении и нуждающихся в экстракции ретенированных моляров. Изучали 24 удалённых зуба, которые помещали в искусственную слюну с добавлением различных сред. Образцы зубов распределены на четыре группы: только искусственная слюна (I, контроль); холекальциферол 1000 МЕ на 100 мл (II); обработка реминерализирующей пастой (III); витамин D и реминерализирующая паста (IV). Исследование структуры эмали зубов проводили методом конфокальной профилометрии с измерением показателей шероховатости Ra и Rp.* ***Результаты.*** *В группе II в присутствии холекальциферола выявлены изменения показателей (Ra, Rp) шероховатости поверхности эмали зубов, в группе III (обработка реминерализирующей пастой) показатели Ra и Rp имели аналогичные цифровые значения. У образцов зубов группы IV в сравнении с группой I отмечена сглаженность шероховатости поверхности эмали, что подтверждают параметры Ra и Rp (p >0,001). Данный эффект можно объяснить синергическим действием холекальциферола и реминерализирующего состава зубной пасты на структуру эмали.* ***Заключение.*** *У лиц с гомозиготным полиморфизмом (A/A) гена VDR установлено достоверно значимое изменение параметров шероховатости эмали зуба (Ra и Rp) при комплексном использовании холекальциферола и реминерализирующей зубной пасты, что связано с выравниванием поверхности за счёт образования гомогенного слоя, состоящего из микрокристаллов.*

Ключевые слова: *полиморфизм; ген VDR; конфокальная профилометрия; реминерализирующая паста; витамин D; холекальциферол.*

Для цитирования:

Тихонова С.Н., Козлова М.В., Горбатова Е.А. Оценка эффективности действия витамина D и реминерализирующей пасты на структуру эмали зубов у лиц с гомозиготным полиморфизмом гена, кодирующего внутриклеточный рецептор витамина D. *Клиническая практика.* 2025;16(2):41–52. doi: 10.17816/clinpract678365 EDN: MJYCOQ

Поступила 10.04.2025 Принята 13.06.2025 Опубликована online 27.06.2025

structural features, which also depend on various genetic variations [2].

There are quite few research works proving that the polymorphism of the gene encoding the intracellular vitamin D receptor (VDR Bsml rs1544410) is the reason of vitamin D deficit [3, 4]. X. Qin et al. [5] have reported the interrelation between low blood cholecalciferol levels and high incidence of dental caries.

Previously, in the individuals with homozygous polymorphism (A/A) of the VDR gene, the experiment on evaluating the enamel structure using the electron scanning microscopy has shown the morphological

https://doi.org/10.17816/clinpract678365

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changes in its surface, resulting from the demineralization process. The results of spectrometric analysis have confirmed the activation of the hard tooth tissues resorption phase, which manifested as an impairment of the microelement content [6]. According to the modern concept from the World Health Organization, one of the most effective strategies for caries prevention is the remineralizing therapy [7–9]. The preventive and therapeutic effects of mineral elements (phosphorus, calcium, fluorine) are based on their inclusion into the crystalline lattice formed by the dental enamel apatites [10].

There are data on the effective endogenous prevention of caries expressed as an additional intake of vitamin D, affecting the calcium-phosphorus metabolism [11].

In patients with homozygous A/A genotype, blood vitamin D deficit takes place, being the reason of impaired calcium metabolism and of developing a dysbalance of bone tissue remodeling [12].

One of the variants of experimental evaluation of the dental enamel structure is the confocal optical profilometry [13], allowing for the assessment of the enamel surface with using the digital methods and for conducting the statistical analysis of the results.

Research aim — to evaluate the effects of using vitamin D and toothpaste with remineralizing components on the structure of dental enamel of the impacted teeth extracted from the individuals with homozygous (A/A) VDR gene polymorphism.

METHODS

Research design

The conducted research is classified as experimental single-center prospective full-design controlled and randomized research (Fig. 1).

SCREENED
200 individuals aged 22–25 years (68 males, 132 females),
studying at the Dentistry Department of the FSBI CPE CSMA in 2023–2025

GENETIC TESTING
Assessment of the *VDR* genotypes (BsmI rs1544410)

Inclusion criteria	Non-inclusion criteria
• patients with detected homozygous (A/A) polymorphism of the *VDR* gene and diagnosed with vitamin D deficit treated with daily intake of cholecalciferol at a dosage of 1000 IU, having the orthodontic indications to extracting the third molars of the mandible, with the diagnosis of "K07.35 Impacted or unerupted teeth" • extracted third mandibular molars with a developed anatomical structure in the abovementioned patients	• screened individuals with the diseases of the gastrointestinal tract, kidneys abnormalities, oncological, autoimmune or endocrine diseases • pregnancy, breastfeeding

36 assessed (16 males, 20 females) with homozygous polymorphism (A/A)
of the *VDR* gene (BsmI rs1544410) and blood vitamin D deficit

24 extracted (according to the orthodontic indications) impacted lower molars
placed into the containers with artificial saliva (4 groups of 6 teeth)

I, control	II	III	IV
only artificial saliva	+ cholecalciferol 1000 IU per 100 ml of solution	+ treatment with remineralizing toothpaste	+ cholecalciferol 1000 IU per 100 ml of solution + treatment with remineralizing toothpaste

CONFOCAL OPTICAL PROFILOMETRY

Fig. 1. Research design.

Within the premises of the Dentistry Department of the Federal State Budgetary Institution of Continuing Professional Education "Central State Medical Academy" (FSBI CPE CSMA), 200 students aged 22–25 years were examined (68 males and 132 females) with conducting the tests for determining the VDR gene polymorphism (BsmI rs1544410).

Conformity criteria

Inclusion criteria: patients with detected homozygous (A/A) polymorphism of the VDR gene and with diagnosed vitamin D deficit despite the daily intake of cholecalciferol at a dosage of 1000 IU, having the orthodontic indications to extracting the third mandibular molars, with the diagnosis of "K07.35 Impacted or unerupted teeth"; the extracted third mandibular molars had a developed anatomical structure in all of the abovementioned patients.

Non-inclusion criteria: individuals with gastrointestinal tract diseases, abnormalities in the kidneys, oncological, autoimmune or endocrine diseases; pregnancy, breastfeeding.

Research facilities

The clinical research itself, the procedures of collecting the genetic material and the extraction of the impacted mandibular molars were arranged within the premises of the Dentistry Department of the FSBI CPE CSMA.

The genetic testing purposed to reveal the polymorphism of the VDR gene encoding the intracellular vitamin D receptor was carried out at the "MyGenetics" National Center of Genetic Tests (Novosibirsk).

The measurements of the dental enamel roughness parameters were conducted using the S Neox confocal optical profilometer (Sensofar, Spain) at the Federal State Budgetary Scientific Institution "Technological Institute for Superhard and Novel Carbon Materials" under the National Research Center "Kurchatov's Institute" (Moscow, Troitsk).

Research duration

The research was carried out during the period from 2023 until 2025.

Medical procedure description

In patients with homozygous polymorphism (A/A) of the VDR gene (n=18), using the conduction and infiltration anesthesia with Ultracaine D-S forte (Sol. Ultracaini forte) at the volume of 1.7 ml, the formation and the delamination of the mucoperiosteal flap was carried out at the level of the third mandibular molar projection, then, using the fissural dental drill, the bony operculum was removed, followed by the extraction of the impacted teeth using the elevator tool. Later on, the mucoperiosteal flap was positioned back to its place and sutured using simple interrupted sutures. A total of 24 teeth were extracted.

Research outcomes

Main research outcome: the extracted teeth were cleared of the soft tissues and placed into the flasks containing the artificial saliva (structurized water; electrolytes: Na^+ 0.3 g/l, K^+ 1 g/l, Ca^{2+} 0.05 g/l, Mg^{2+} 0.01 g/l, Cl^- 0.1 g/l, $(PO)_4^{3-}$ 0.1 g/l; organic substances: 0.5% carboxymethylcellulose and alginic acid, urea). The dental samples in quantities of six were placed into four flasks according to the following groups: group I contained only the artificial saliva solution (hereinafter referred to as the Control group); in group II, the solution of artificial saliva was supplemented with cholecalciferol at a concentration of 1000 IU/100 ml; in group III, into the enamel of the extracted teeth, by means of the electric tooth brush, toothpaste was rubbed in for 2 minutes twice daily (following the 10 hours interval), with the paste containing the microRepair microcrystals; in group IV, the extracted teeth processed with the toothpaste according to group III protocol, were placed into the flask containing the artificial saliva with cholecalciferol at a concentration of 1000 IU/100 ml.

The flasks were stored at the incubation chamber at 37°C for 72 hours, after which the teeth were taken out and washed with water for 60 seconds.

Methods for registration of outcomes

The measurements in the dental enamel roughness parameters were made at the contact surface of the molars at the level of the tooth equator using the diagonal and the horizontal profiles by means of the S Neox confocal optical profilometer (Sensofar, Spain). The three-dimensional images of the surface were obtained with the ×50 objective lens (vision field dimensions 351×264 μm, resolution 0.13 μm) with using the LED (Light Emitting Diode) light source at various wavelengths: red (630 nm), green (530 nm), blue (460 nm) and white.

The digital processing of the dental enamel surface scans was conducted using the Senso SCAN and Gwyddion software products.

The procedures of evaluating the roughness parameters of the enamel surface were carried out in

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accordance with the State Industry Standard (GOST) R ISO 25178-2-2014[1], regulated by the State Industry Standard (GOST) 2789-73. The automatic data calculations were done using the Sensofar software (in accordance with ISO 4287).

The following parameters were evaluated within the ranges of the basic length, allowing for interpreting the changes in the dental enamel surface roughness at the stages of the experiment: Ra (average roughness) — the arithmetic mean of the deviations in the estimated profile; Rp (maximum peak height) — the maximal peak height within the profile.

Statistical analysis

For the statistical processing of the results and for describing the data, the Statistica 13.3 (StatSoft Inc.) and the GraphPad Prism 9 version 9.4.1 software products were used. The mean values of the parameters were provided as M±m, where M is the mean value and m is the error of the mean. The statistically significant differences taken into account were the ones with the significance level (*p*) not exceeding 0.05.

RESULTS

Research sample (participants)

Within the premises of the Dentistry Department of the FSBI CPE CSMA, a total of 200 students aged 22–25 years (68 males and 132 females) were screened and underwent testing purposed to determine the polymorphism of the VDR gene (Bsml rs1544410).

Based on the results of genetic testing, the group with homozygous A/A allele of the VDR gene consisted of 36 individuals (16 males and 20 females) with diagnosed vitamin D deficit (blood serum concentration was 9.04±2.02 ng/ml). From this group, 18 individuals were selected (7 males, 11 females), in which, due to the presence of orthodontic indications, an extraction of the third mandibular molars was carried out (diagnosis: “K07.35 Impacted or unerupted teeth”).

Primary findings

In the individuals with homozygous polymorphism (A/A) of VDR gene, the microstructure of the dental enamel samples stored in the artificial saliva solution (group I, control) had changes in the structural profile. The three-dimensional image of the surface of dental sample, provided in Fig. 2, had a characteristic pattern of numerous peak-like elevations above the baseline surface, presented as an inhomogeneous plane of predominantly red color. Fig. 3 shows the graphic profile of such surface, having a high rate of alternating peaks and valleys. After the digital processing of the obtained data, the Ra value was determined, which equals 0.436±0.052 upon measuring in the diagonal direction and 0.308±0.172 for the horizontal direction (Fig. 4), along with the values of the maximal height variation of the Rp — 1.421±0.16 and 1.440±0.46, respectively (Fig. 5).

[1] National Standard of the Russian Federation. GOST R ISO 25178-2-2014. The geometric characteristics of the products (GPS). Access mode: https://docs.cntd.ru/document/1200116349?ysclid=mc1xy4b8js53577141

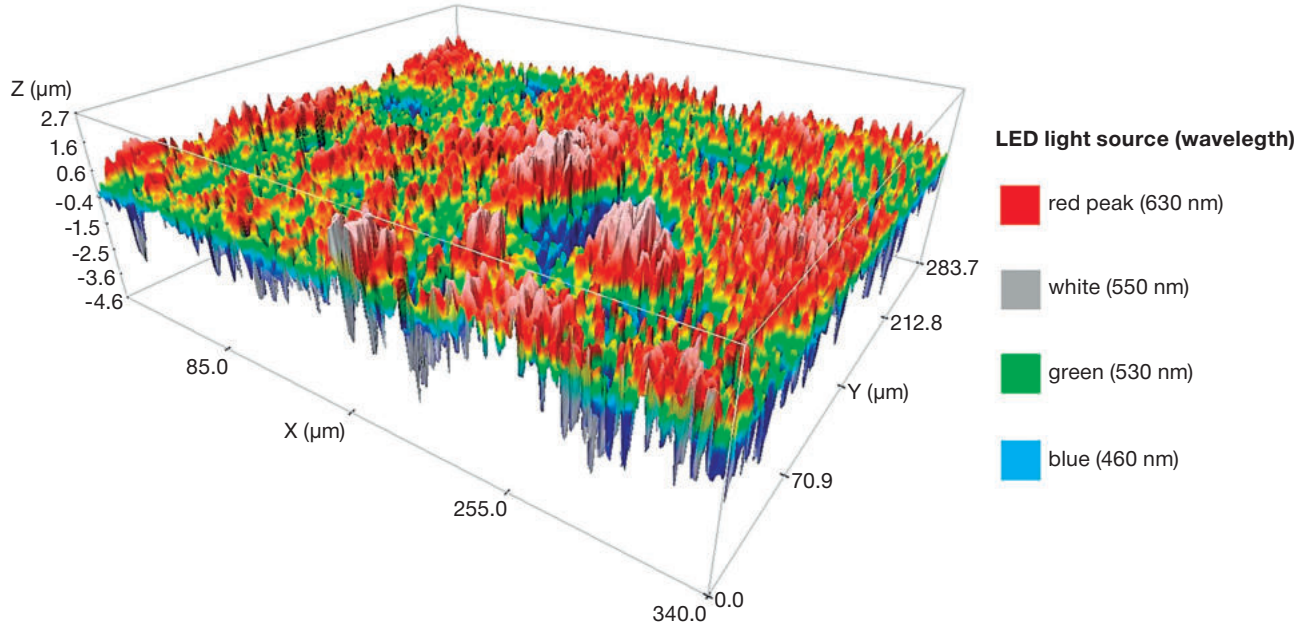

Fig. 2. Three-dimensional image of the enamel surface sample from group I.

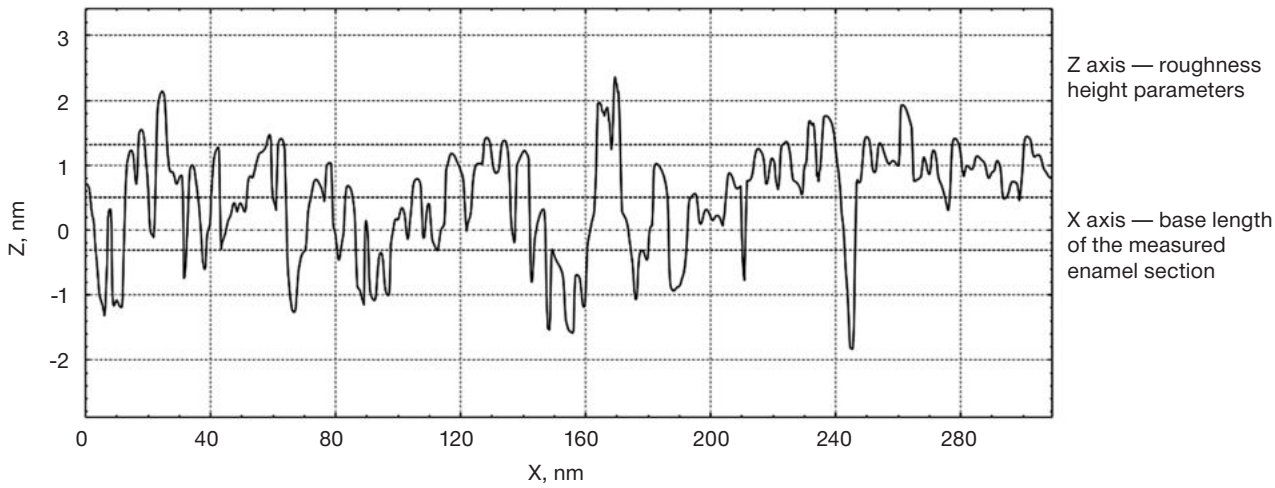

Fig. 3. Enamel surface profile of the sample from group I.

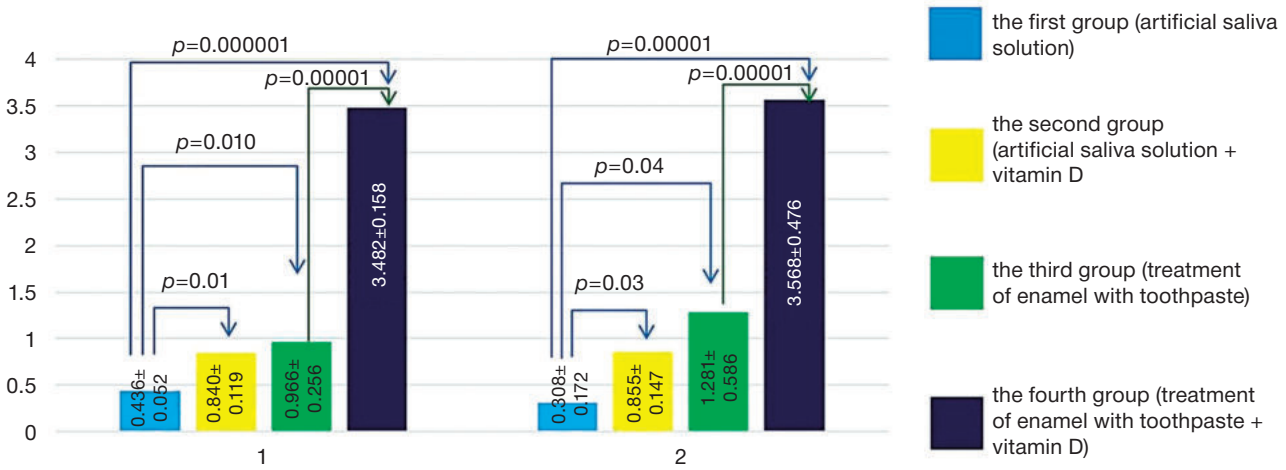

Fig. 4. Ra parameter chart (the arithmetic mean of the deviations for the estimated profile: 1 — diagonal measurement; 2 — horizontal measurement).

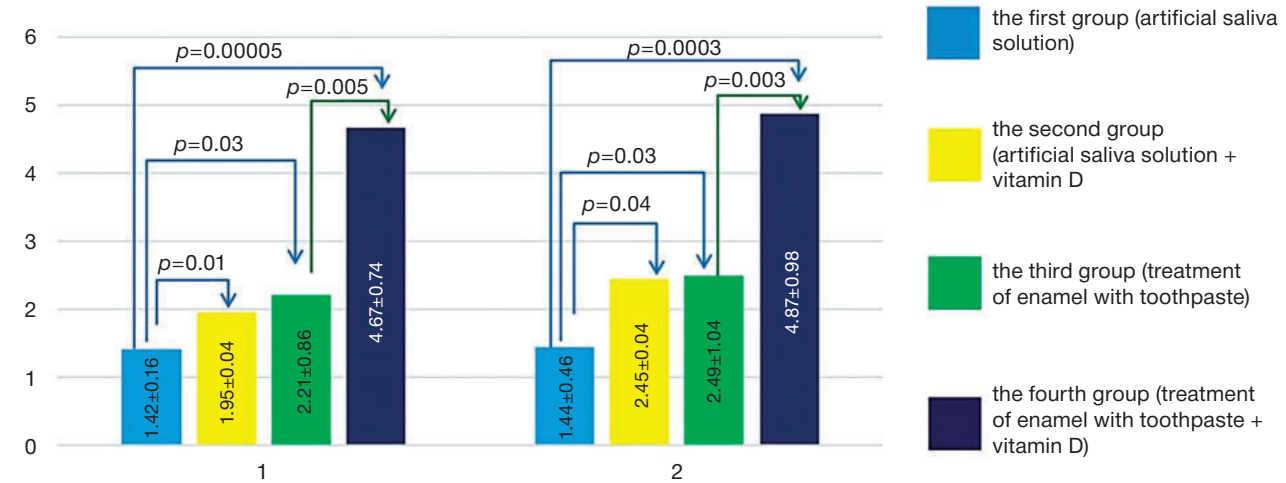

Fig. 5. Rp parameter chart (maximum height of the profile peak: 1 — diagonal measurement; 2 — horizontal measurement).

https://doi.org/10.17816/clinpract678365

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The enamel surface in the extracted impacted teeth from the group II with an addition of 1000 IU of cholecalciferol to the artificial saliva solution, upon the analysis of its three-dimensional images, had an enlarged area of the green and blue-colored fields (Fig. 6). The graphic representation of the height peaks has shown a moderate variation between the highest and the lowest points of the roughness line graph (Fig. 7). After the digital evaluation of the surface profile, the Ra parameter was 0.840±0.119 for the diagonal measurement and 0.855±0.147 for the horizontal one (see Fig. 4), the Rp criterion has shown the values of 1.95±0.04 and 2.45±0.04 (see Fig. 5).

The surface pattern of dental samples after rubbing the toothpaste with remineralizing compound in group III was visualized in the three-dimensional images as the predominance of colored areas of green and blue spectrum as compared to the moderately expressed red-colored areas (Fig. 8). The distribution of colors had intensive optical borders. The graphic representation of the surface of the enamel treated with the toothpaste, was observed as a wave-like line, in which foci of smoothened peak profile was alternating with the peak-like ones (Fig. 9). The values of roughness were the following: Ra 0.966±0.256 (diagonal measurement) and 1.281±0.586 (horizontal measurement) (see Fig. 4), while the height variation (Rp) was 2.21±0.86 and 2.49±0.41, respectively (see Fig. 5).

In group IV, after processing with the toothpaste having a remineralizing effect and after an addition of vitamin D (1000 IU per 100 ml), the three-dimensional images of the surface looked structurized, without any

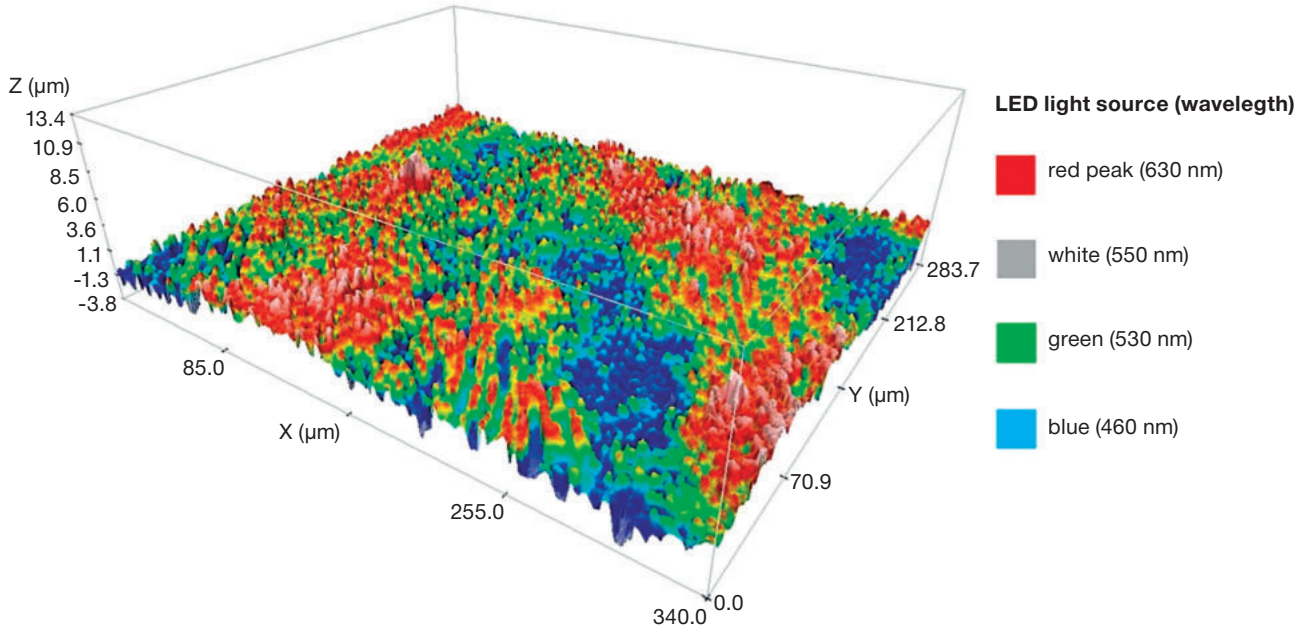

Fig. 6. Three-dimensional image of the dental enamel surface sample from group II.

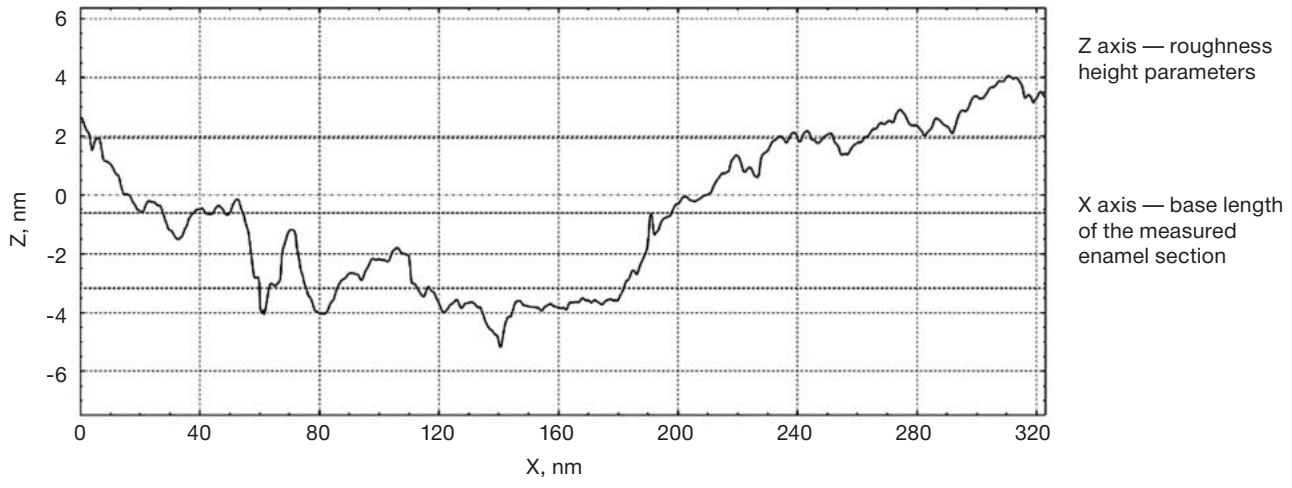

Fig. 7. Enamel surface profile of the sample from group II.

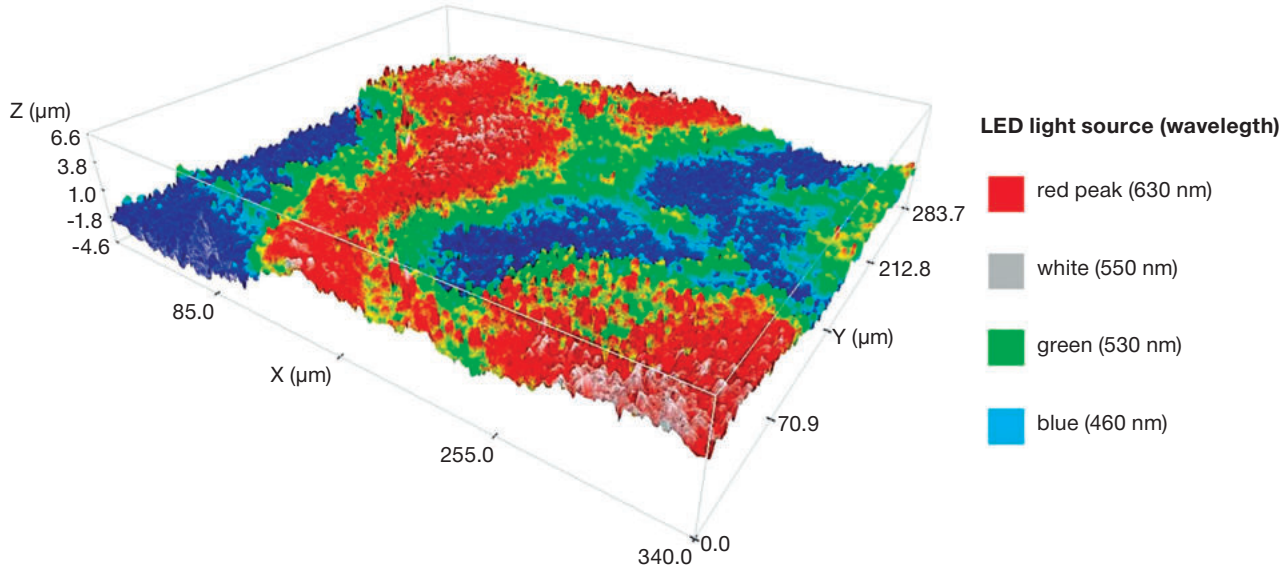

Fig. 8. Three-dimensional image of the dental enamel surface sample from group III.

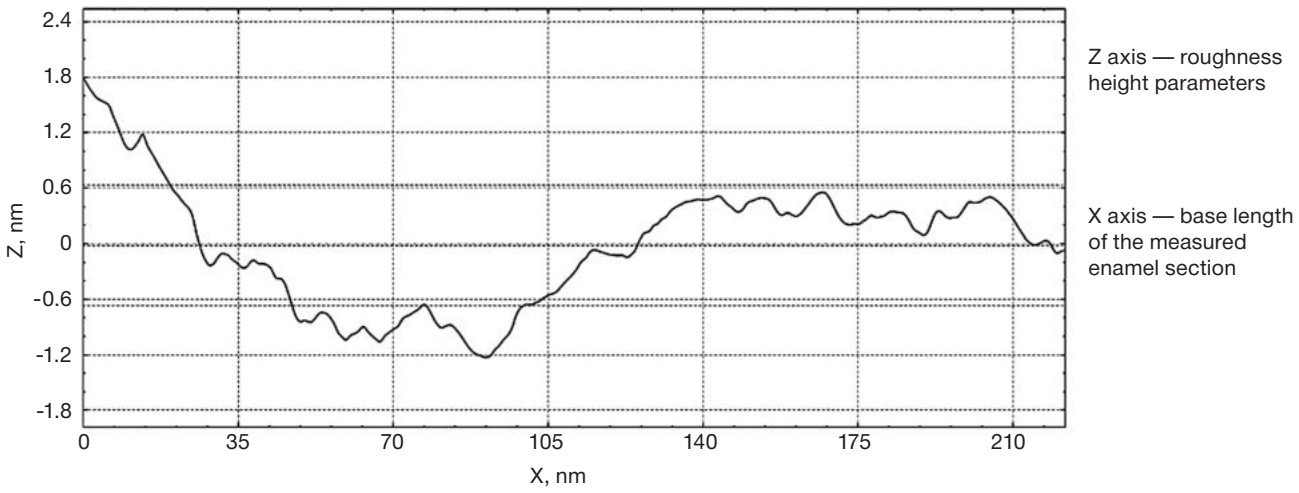

Fig. 9. Enamel surface profile of the sample from group III.

sharp peaks or valleys, also having a clear geometry of colored zones (Fig. 10). The profile graph did not have a peak-like gradient of graphic height, while the enamel surface has gained quite a smooth pattern with no signs of porous structure (Fig. 11). The roughness parameter Ra had the following values: diagonal measurement 3.482±0.158, horizontal - 3.568±0.476, Rp — 4.67±0.74 and 4.87±0.98, respectively (see Fig. 4, 5).

DISCUSSION

In all the groups, upon analyzing the results of measurements at the horizontal and diagonal directions, we have obtained the parameter values with no significant difference, which indicated the significance of data measured for the surface of the object having a volumetric non-linear shape — the equatorial zone of the contact surface of the tooth.

In patients with homozygous polymorphism (A/A) in the VDR gene, when evaluating the results of confocal profilometry, we have found that in group II (teeth contained in the solution of artificial saliva and vitamin D), changes have developed in the roughness of the enamel surface of the extracted teeth. An elevation of the values for the parameters Ra (1.4-fold; p=0.01) and Rp (2-fold; p=0.01) comparing to the group I (control) indicated the smoothness of the surface profile (see Fig. 4, 5). In the three-dimensional images, the additionally visualized findings included an enlargement of the areas colored green and blue (see Fig. 6). It can be suggested that this transformation

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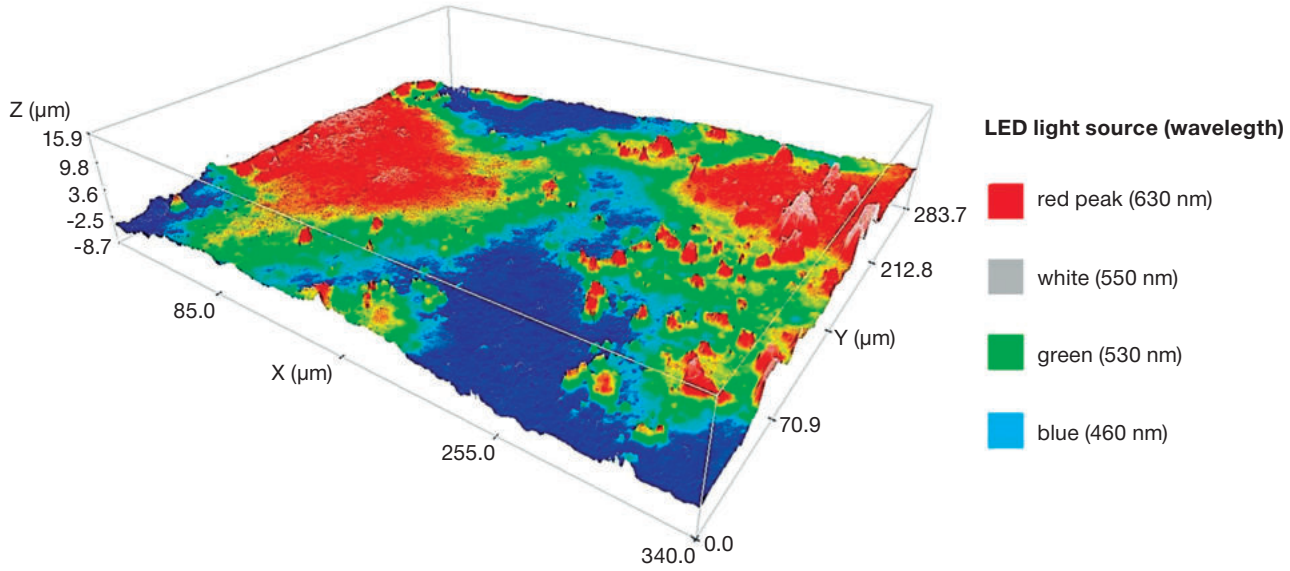

Fig. 10. Three-dimensional image of the dental enamel surface sample from group IV.

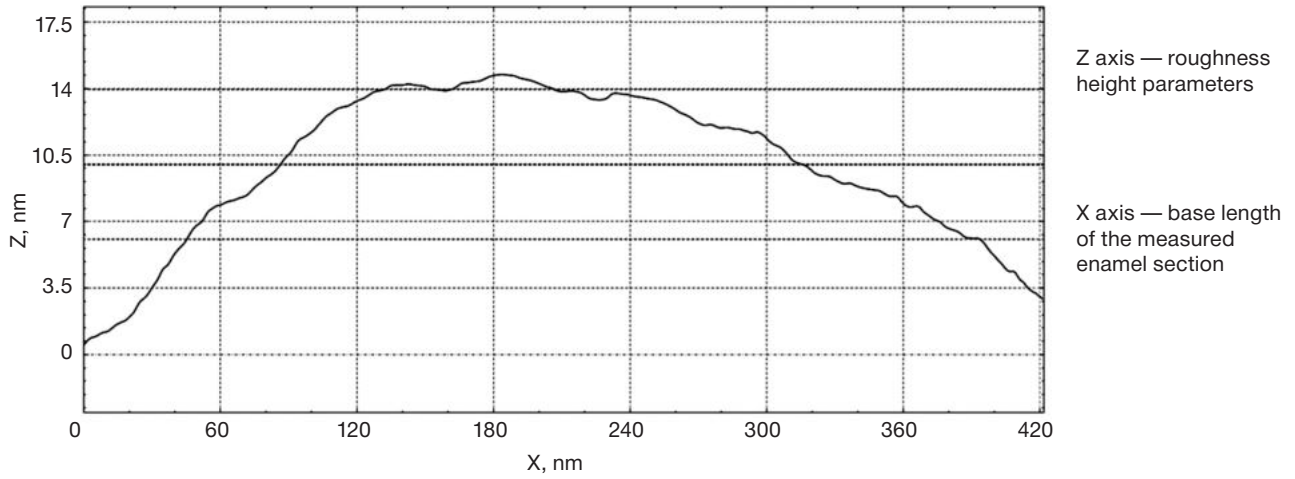

Fig. 11. Enamel surface profile of the sample from group IV.

of the enamel surface is related to the formation of mineralization zones due to the sedimentation of calcium-phosphate compounds from the artificial saliva solution in the presence of vitamin D.

The results in groups II (addition of cholecalciferol to the artificial saliva solution) and III (processing the enamel of the extracted teeth with remineralizing toothpaste) had significant changes in the roughness parameters comparing to the group I (control).

Upon comparing the Ra and Rp digital parameters between groups II and III, statistically insignificant differences were revealed: the presence of cholecalciferol in the artificial saliva or processing with remineralizing toothpaste have demonstrated similar digital values of roughness.

As for the samples from group IV (extracted teeth contained in the artificial saliva solution with vitamin D and processed with remineralizing toothpaste), they have demonstrated significant changes in the roughness of the enamel surface, which was confirmed by an increase in the values of Ra (8-fold; p=0.000001) and Rp (3-fold; p=0.00005) comparing to the control group I (see Fig. 4, 5). The obtained results are determined by the synergetic effects of vitamin D and microRepair microcrystals contained in the toothpaste.

This research points out that, among the individuals carrying the homozygous polymorphism (A/A) of the VDR gene, the combined use of remineralizing toothpaste and cholecalciferol promotes to the

improvement in the structure of the dental enamel and increases the level of its resistance.

CONCLUSION

In patients with homozygous polymorphism (A/A) of the VDR gene, upon containing the extracted impacted teeth in the solution of artificial saliva with further addition of cholecalciferol at a concentration of 1000 IU/100 ml and upon treating the enamel with remineralizing toothpaste, significant changes were shown for the Ra (p=0.000001) and Rp (p=0.00005) parameters. The increase of the values of these parameters results in a decrease in the pronounced roughness of the dental enamel, which is related to the formation (in the presence of vitamin D evenly distributed along the surface) of the homogeneous layer consisting of microRepair microcrystals.

The obtained results can be used for developing the program of preventing dental caries related to the impaired mineral metabolism caused by the homozygous polymorphism (A/A) of the VDR gene among the patients, which are in the high risk group.

ADDITIONAL INFORMATION

Author contributions. *S.N. Tikhonova:* collection and analysis of literary sources, conducting the experimental part of the study, preparing and writing the text of the article; *M.V. Kozlova:* idea and design of the study, surgical treatment of patients participating in the study, editing the text of the article; *E.A. Gorbatova:* analysis of literary sources, editing the text of the article. Thereby, all authors provided approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements. The authors express gratitude to the employees of “MyGenetics” National Center of Genetic Tests” LLC (Novosibirsk, Russia): to the Head of the Development Department V.V. Polunovskiy, to the Senior Specialist O.V. Chedulova for arranging the analysis of genetic material and for the interpretation of the obtained data.

The authors express gratitude to the Senior Research Associate of the Laboratory of the FSBSI “Technological Institute for Superhard and Novel Carbon Materials” under the National Research Center “Kurchatov’s Institute” (Moscow, Troitsk, Russia) E.V. Gladkikh for the provision of equipment and for the help in arranging the experimental part of the research.

Ethics approval. All the patients enrolled into the research have signed the voluntary consents for the conduction of all the medical procedures, according to the Decree issued by the Ministry of Health and Social Development of the Russian Federation No. 390n dated 23.04.2012, in accordance with the Article 20 of the Federal Law No. 323 dated 21.11.2011. The research protocol was approved by the local Ethics Committee of the FSBI CPE CSMA, extract from the session protocol No. 1, dated 07.02.2023.

Funding sources. No funding.

Disclosure of interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of originality. The authors did not utilize previously published information (text, illustrations, data) in conducting the research and creating this paper.

Data availability statement. The editorial policy regarding data sharing does not apply to this work, data can be published as open access.

Generative AI. Generative AI technologies were not used for this article creation.

Provenance and peer-review. This paper was submitted to the journal on an initiative basis and reviewed according to the usual procedure. Two external reviewers and the scientific editor of the publication participated in the review.

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