UDK 661.31

MORPHOLOGY OF THE HUMAN DENTAL ENAMEL

© 2014 P. V. Seredin[1], Y. A. Ippolitov[2], I. Y. Ippolitov[2], D. L. Goloshchapov[1], A. S. Lenshin[1], A. A. Al-Zubaidi[1]

[1]Voronezh State University, Universitetskaya sq., 1, 394006 Voronezh, Russia
[2]Voronezh State Medical Academy, Studencheskaya st., 10, 394000 Voronezh, Russia
e-mail: paul@phys.vsu.ru

Received 09.09.2013

Abstract. Enamel morphological formations participating in hard tooth tissues metabolic processes were studied by scanning electron microscopy, X-ray spectroscopic micro- and quantitative analyses and histochemical methods of examination.

Keywords: tissue, enamel, biopolymers.

INTRODUCTION

Enamel is the most mineralized tissue of the living organism. It includes 95 % mineral substances (mainly hydroxyapatite, carbonated apatite, fluorapatite etc.), 1.2 % — organic compounds, 3.8 % is connected with water bound within the crystals, organic compounds and unbound water [1].

Dental enamel is known to be the hardest human tissue. This allows it to withstand the impact of large mechanical loads during the fulfillment of the tooth's functions. It is well known that enamel consists by more than 90 % of the mineral compounds (mainly hydroxyapatite — HAP [$Ca_{10}(PO_4)_6(OH)_2$], fluorapatitea [$Ca_{10}(PO_4)_6F_2$], carbonated apatite et al.), by 1.2 % — of organic compounds and referred to the bound water in the crystals and organic components as well as free water [2—4].

The composition of organic components in the mature enamel is known rather tentatively. Jenkins G. N. provides the following numerical data on the content of organic substances in the enamel of premolars and molars (percentage-wise relative to dry solid matter) — insoluble proteins — 0.3—0.4 %, soluble proteins — 0.05 %, fat — 0.6 %, citrates — 0.1 % [5]. The most widespread proteins, about 90 % of all the organic fraction are represented by hydrophobic proteins (amelogenins) enriched with amino acids and 10 % are acid proteins — enamelins [6].

Enamel does not involve cells and is not capable of regenerating damage. However, an exchange of mineral ions permanently takes place within enamel. These ions are provided from the underlying dental tissues (dentin, pulp) and from saliva [7, 8]. Simultaneously with ion provision (remineralization) ion removal from enamel also takes place (demineralization). These processes are in continuous equilibrium. Its shift towards one of the processes depends on a number of factors, including the content of micro- and macroelements in the saliva, pH value within the oral cavity and on the tooth surface [1]. The degree of enamel penetrability is not the same during different periods of the odontogenesis. It is reduced in the following series: enamel of non-erupted tooth → enamel of deciduous tooth → enamel of the permanent tooth of young person → enamel of the permanent tooth of the aged person.

The mechanism of penetration of the organic compounds into dental enamel is not yet clear in details. First of all it should be noted the presence of special morphological formations intended for providing of organic compounds and mineral ions into the dental enamel — these are lamellas. Moreover, enamel plates, bundles and spindles — parts of enamel involving poorly calcified enamel prisms and interpism substance was observed and described, where rather considerable concentration of proteins with high molecular mass similar to enamelin protein was revealed [7].

The role of organic composition of the dental enamel matrix is actively investigated including its state in the caries initiation process [9—11].

Organic matrix bound to the crystals that provides for growth and orientation during the formation of enamel is almost completely lost during maturation of dental enamel. It is preserved in the form of the finest 3D protein grid while its wires are arranged between the prisms. Recent investigations provided new data

on the nature and functions of the organic matrix of the enamel.

It was confirmed that its most important role is the stabilization of the buffer system providing free calcium ions in this system [12]. It should be noted that organic components of the enamel matrix has been so far studied to a less extent that its mineral phase. Calcium-binding protein, which is capable of depositing in the neutral medium in the presence of calcium ions, is considered to be functional elementary block of the organic matrix in the enamel. The calcium-binding protein of the enamel and acid-insoluble protein both determine the orientation of the crystals in enamel prisms and its structure.

The surface of the dental enamel under examination is characterized by microrelief formed with pericamatia and hollows in the form of pits that can be observed with the use of scanning electron microscope.

The appearance of the new technologies allows us to study the composition of organic structure in the hard dental tissues at a qualitatively new level [13, 14].

OBJECTIVE OF THE INVESTIGATIONS, MATERIALS AND METHODS

The study of morphological formations and substances of the protein nature in the human dental enamel.

Using scanning electron microscopy (SEM) in the secondary electron emission mode as well as X-ray spectral microchemical quantitative analysis (XRMA), dental enamel of the teeth extracted according to orthodontic indications for the patients at aged 17—25 years was investigated. The surface of enamel for each of 50 teeth was examined at magnification of 800 to 4000 times with the use of low-vacuum electron microscope GEOL GSM — 6380 LV produced in Japan.

The use of SEM allowed attaining a high resolution without the preliminary treatment of the objects (meaning teeth) with gold or carbon thus providing the possibility to reliably estimate the quantitative microchemical analysis of microstructures and formations on the enamel surface with the use of XRMA. The images and results of the study were registered using digital technique.

For the first time a hypothesis regarding the presence of the "cation protein" (CP) and a set of the constituent amino acids was tested in a series of the histochemical experiments. Taking into account the histo- and cytogenesis of the dental structures and the previously obtained results based on the study of the multi-layered partially keratinizing pavement epithelium of the mucous tunic in the oral cavity the investigations were performed based on the original technique of preparation of thin paraffin sections made of the whole teeth as well as the modified histochemical technology of CP identification with the use of bromphenol blue reagent [12, 15, 16].

Histochemical preparations were obtained from the teeth, extracted according to the orthodontic indications. Quantitative investigations were performed at the "Microtels-4" facility where digitization of the micro-TV image was made within the limits of certain selected regions; in addition, extinction values were calculated point by point. Screening operations were performed in pseudo-color and then the image was displayed. The sensed regions involving the necessary structures were limited manually by square technique operation, the calculations of statistical tables were made automatically after the selection of the measured displayed regions. To solve the problem of the representative sampling the accumulated average technique was applied. Ten premolars of the human upper jaw was studied (the sampling was applied for the descriptive analysis and conformity/discernibility arraying by Wilcoxon method). Statistical processing was performed with the use of the STX routine, the sampling length was 100 values.

The presence of CP can be identified by the binding of bromphenol blue molecules with the structures of decalcified tooth at pH value 8.2. Control reactions were performed with the use of trypan and chloride sulfoxide for the protein depolymerization and blocking of the carboxyl groups in the "marker" amino acids and CP. After performing the shic-reaction with a periodate oxidation the concentration level of the neutral glycoproteins (NGP) in the hard dental tissues was determined as well as the concentration level of the acidic non-sulfated glycoprotein — hyaluronic acid (HA) using Muller method just as characteristic biopolymers of the "tissue barrier".

RESULTS OF INVESTIGATIONS

The enamel surface on the electron scanning images of the teeth fragments is represented by characteristic formations in the form of roughness and small hollows (pits) with a diameter of 4—6 μm and a depth of 0.5—3 μm. In addition, microcracks and elevations in the enamel microrelief is also represented by the clefts in the form of holes as if passing into tubules with a diameter up to 2 μm thus providing a honeycomb structure. A targeted quantitative microchemical analysis made it possible to determine chemical element composition on the surface of enamel and in the outfall of the enamel hollow (see electron images

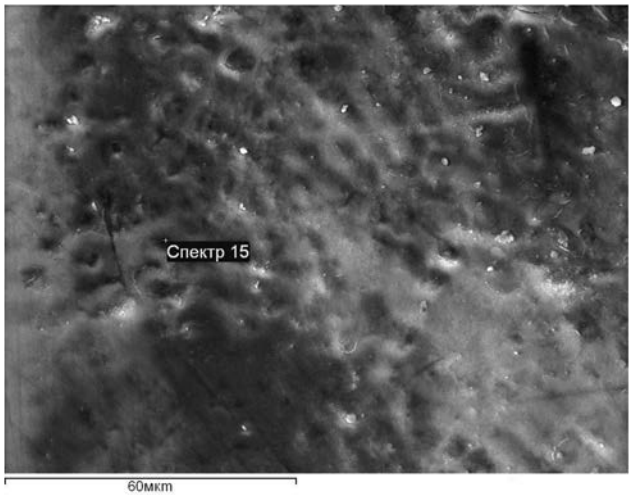

Fig. 1. Electron image of the enamel surface. Magnification by 1500 times

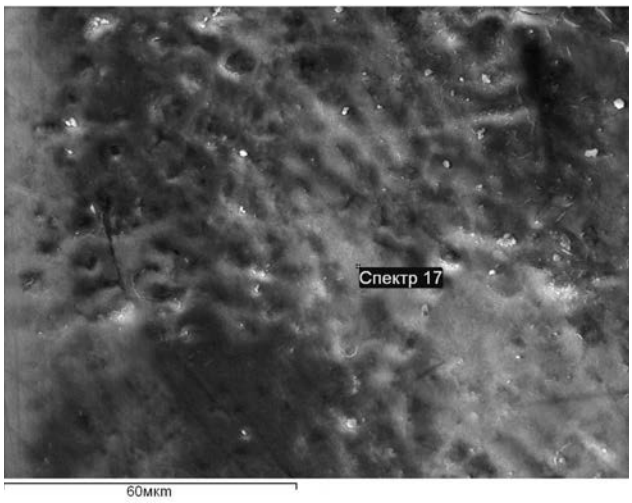

Fig. 2. Electron image of the enamel surface. Magnification by 1500 times

№ 1, 2, 3, 4 and the corresponding Tab. № 1, 2, 3, 4 including the data on the quantitative analysis of microelements). Electron images of № 3 and 4 represent enamel splits where one can clearly observe crystal-shaped structures along the surface of the enamel prisms as well as the porous surface reminiscent of the hollows in the form of tubules. Results of the quantitative analysis for the empty area in the form of tubules are presented in the Tab. shown under the electron image.

Quantitative analysis showed an increase of the weight percent of carbon in the enamel tubule meaning an increased content of organic component in this kind of morphological formation while the weight percent of calcium, phosphorus, chlorine and oxygen is higher in the apatite crystal of the enamel surface than in the tubule of the enamel crystal.

Protein is detected in histochemical micropreparations of all dental parts and it is connected with the structure of tubules in dentin and enamel and is revealed as a homogeneous coloration

CP content is rather high in the enamel structures — $0.3559 + 0.01$, $\sigma = 0.119$, while in the nearest dentin the corresponding values were of $0.1528 + 0.004$, $\sigma = 0.045$.

Results of microdensitometry can become a basis for the hypothesis on the ways of formation protective dental media, while their pathogenetic significance for the understanding of appearance and development of the carious process is yet unknown.

At the same time investigations into the diagnostics of the statistical model basing on descriptive analysis with the use of distribution diagram obtained on the Pearson plane.

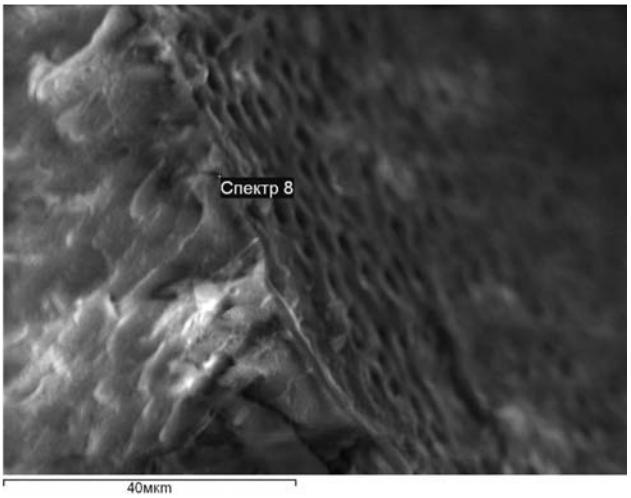

Fig. 3. Electron image of the split near the surface of enamel. Magnification by 2000 times

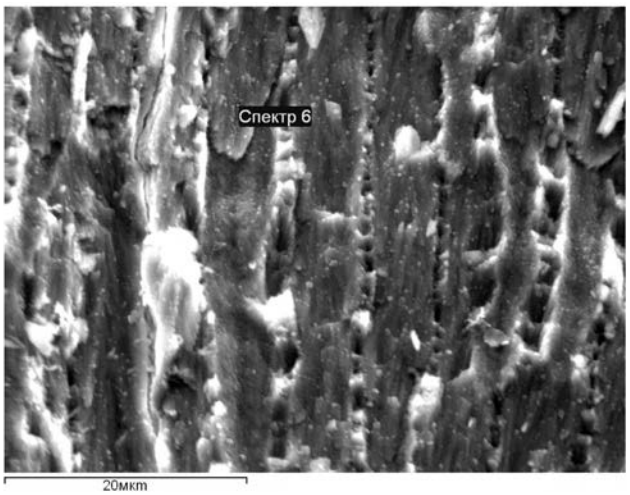

Fig. 4. Electron image of enamel split. Magnification by 4000 times

Table 1. Quantitative microchemical analysis in the region of enamel tubule

Element	Weight %	Atomic %
C	20.54	31.60
O	34.71	40.10
P	19.12	11.41
Cl	0.67	0.35
Ca	35.88	16.54
In total	110.92	

Table 2. Quantitative microchemical analysis in the region of enamel crystal

Element	Weight%	Atomic%
C	10.24	14.75
O	51.75	55.99
Na	0.69	0.52
P	21.10	11.79
Cl	0.81	0.40
Ca	38.31	16.54
In total	122.91	

The statistical model for CP of enamel is described by a simple β-distribution.

Moreover, after performing of the shic-teaction with a periodate oxidation a higher level of NGP was determined in the enamel adjacent to dentine than on the surface of the enamel.

HA — acidic non-sulfated glycoprotein is distinguished by rather high variations in topochemisty and composition. High values of reactivity of this glycoprotein are detected in the interprism substance although an inhomogeneity of the distribution for this colorant can be observed under transition from enamel to dentin.

If one accounts for the role of carbohydrate-protein biopolymers and the substances of a protein nature as a protective factor from bacteria and the products of their metabolism for the multi-layered pavement epithelium of the human organism then it seems possible to assume the realization of a similar function in the hard dental tissues as well; then the microscopic areas filled with biopolymers form a common diffuse system of the mineral ions exchange in the human tooth.

CONCLUSION

Carbohydrate-protein biopolymers and the substances of a protein nature are detected in the interprism areas of dental enamel playing an important role in the metabolic processes taking place in the human dental enamel forming physiological barrier for microorganisms and the products of their vital activity.

It should be noted that a higher content of organic component is obtained for the demineralization process of the enamel crystal matrix, and plays an opposite role and promotes caries [9—11]. Thus maintaining the carbon-protein balance is the key to the health of a hard tooth.

Table 3. Quantitative microchemical analysis in the region of the enamel tubule

Element	Weight %	Atomic %
C	19.08	30.70
O	38.54	46.56
Na	0.41	0.34
P	14.91	9.30
Cl	0.58	0.32
Ca	26.47	12.77
In total	100.00	

Table 4. Quantitative microchemical analysis in the region of the enamel tubule

Element	Weight %	Atomic %
C	30.23	51.62
Na	0.62	0.55
Mg	0.28	0.23
P	19.06	12.62
Cl	0.43	0.25
Ca	37.11	18.99
O	12.27	15.73
In total	100.00	

ACKNOWLEDGEMENTS:

The work was supported by the Russian Foundation for Basic Research. Projects #13—02—97500 r_centr_a and grant PSR-MG/17—3.

REFERENCES

1. *Borovskii E. V., Leontiev V. K.* // M.: Meditsina, 1991. 304 p.

2. *Goloshchapov D. L., Kashkarov V. M., Rumyantseva N. A., Seredin P. V., et al.* // Ceramics International. 2013. V. 39. I. 4. P. 4539—4549.

3. *Goloshchapov D. L., Kashkarov V. M., Rumyantseva N. A., et al.* // Kondensirovannye sredy i mezhfaznye granitsy. (CONDENSED MATTER AND INTERPHASES). 2011. T. 13. № 4. S. 427—441.

4. *Kashkarov V. M., Goloshchapov D. L., Rumyantseva A. N., Seredin P. V., et al.* // J. of Surface Investigation. X-ray, Synchrotron and Neutron Techniques. 2011. V. 5. I. 6. P. 1162—1167.

5. *Jenkins G. N.* // 4-th ed. Oxford. 1978. 599 p.

6. *Goldberg M., et al.* // Int. J. Dev. Biol. 1995. V. 39. P. 93—110.

7. *Bykov V. L.* / Histology and embryology of the human oral cavity organs: tutorial // SPb. 1998. 248 p.

8. *Bergman G.* // Arch. Oral. Biol. 1963. V. 8. P. 233—235.

9. *Seredin P.l, Kashkarov V., Lukin A.* // J. Synchrotron Rad. 2013. V. 20. P. 705—710.

10. *Seredin P. V., Lukin A. N., Ippolitov Yu. A. //* Nauchnye vedomosti BelGU. Seriya Meditsina. Farmatsiya. 2011. № 16 (111). V. 15/1 C. 104—109.

11. *Ippolitov Yu.A., Lukin A. N., Seredin P. V.* // Vestnik novykh meditsinskikh tekhnology. 2012. T. 19. № 2. S. 343—346.

12. *Shubich M. G.* // Archives of anatomy, histology and embryology. 1973. V. 65. № 8. P. 8.

13. *Leontiev V. K., et al.* // Proceedings of the conference devoted to the memory of Prof. A. A. Panikorovskii. 2001. P. 59—62.

14. *Kunin A. A., et al.* // Contemporary methods of prevention, diagnostics and treatment of the most important diseases: materials of the program-targeted investigations devoted to the 80-th anniversary of VSMA. Voronezh, 1998. P. 101—102.

15. *Bykov E. G.* // The way of histidine detection: Innovatory № 1155 on 10.05.1989. Voronezh: VSMI. 1989.

16. *Merkulov G. A.* // L.: Medgiz, 1961. 340 p.

Середин Павел Владимирович — д. ф.-м. н., с. н. с., кафедра физики твердого тела и наноструктур, Воронежский государственный университет; e-mail: paul@phys.vsu.ru

Голощапов Дмитрий Леонидович — к. ф.-м. н., ведущий инженер, кафедра физики твердого тела и наноструктур, Воронежский государственный университет

Леньшин Александр Сергеевич — к. ф.-м. н., с. н. с., кафедра физики твердого тела и наноструктур, Воронежский государственный университет; e-mail: lenshinas@phys.vsu.ru

Ипполитов Юрий Алексеевич — д. м. н., доцент, кафедра терапевтической стоматологии, Воронежская государственная медицинская академия; e-mail: stomat@vmail.ru

Ипполитов Иван Юрьевич — аспирант, кафедра терапевтической стоматологии, Воронежская государственная медицинская академия; e-mail: stomat@vmail.ru

Аль Зубайди Асаад Абдулхусейн — аспирант, кафедра физики твердого тела и наноструктур, Воронежский государственный университет

Seredin Pavel V. — Dr. Sci. (Phys.-Math.), Senior Researcher, Department of Solid State Physic and Nanostructures, Voronezh State University; e-mail: paul@phys.vsu.ru

Goloshchapov Dmitry L. — Cand. Sci. (Phys.-Math.), Chief Engineer, Department of Solid State Physic and Nanostructures, Voronezh State University

Lenshin Aleksandr S. — Cand. Sci. (Phys.-Math.), Senior Researcher, Department of Solid State Physic and Nanostructures, Voronezh State University; e-mail: lenshinas@phys.vsu.ru

Ippolitov Yuri A. — Dr. Sci. (Medical), Senior Researcher, Associate Professor, Department of Dental Research, Voronezh State Medical Academy; e-mail: stomat@vmail.ru

Ippolitov Ivan Yu. — postgraduate student, Department of Dental Research, Voronezh State Medical Academy; e-mail: stomat@vmail.ru

A. A. Al-Zubaidi — postgraduate student, Department of Solid State Physic and Nanostructures, Voronezh State University