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Microcomputed Tomography in Qualitative and Quantitative Evaluation of Dental Enamel Demineralization

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ABSTRACT

This paper focused on employing microcomputed tomography (micro-CT) to improve the identification of early caries affecting permanent molars' chewing surface in children undergoing eruption. A high-resolution microCT system Skyscan 1176 was used to examine 83 molars extracted (for orthodontic reasons) from children aged 8-11. Out of the total number of the teeth, the following groups were classified – teeth bearing no signs of demineralization, and teeth with carious lesions coming to the stages of white, light brown, brown, and black spots. Reconstructed 2D and 3D images were used to identify areas in the outer, middle and inner third of the enamel, which were followed with calculation of the X-ray density average values using the CTvox software. Given the microCT outcomes, the following descending-order sequence was identified in terms of the optical density factors: healthy enamel – white spot caries – light-brown spot caries – brown spot caries – black spot caries.

Keywords: Microcomputed Tomography, Child Population, Mineral Optical Density, Fissural Dental Caries, Enamel Demineralization.

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INTRODUCTION

According to the reports by the World Health Organization (WHO, 1998), carious lesions in the child population remain one of the key dentistry issues nowadays, which is due to nearly total prevalence of this pathology. In the Russian Federation, the occurrence of permanent teeth carious lesions among schoolers aged 12 varied within the range of 80.7% – 100% depending on the area, whereas the intensity – following the WHO classification – appeared in almost all stages, from low to very high. During the period of permanent teeth eruption, caries is the most often localized in the first molars, which accounts for about 57.4% of the total number of the teeth erupting with Degree 1 of caries intensity, and for 98.6% – in children with Degree 3 of caries intensity [1-5].

The final (tertiary) enamel mineralization occurs after eruption, and is the most intensive through the first year following the tooth crown rising into the oral cavity. Further on, phosphorus and calcium ions penetrating the enamel from the supersaturated solution of hydroxyapatite in saliva, which is slightly alkaline, deliver complete (final) mineralization and high resistance to the effect of organic acids and that of oral microbial flora waste products [6-9].

Of the key properties determining enamel's capacity for mineralization (remineralization), the following functional features have been identified: low rate of metabolic activities in the enamel; high ion permeability for mineral components, amino acids, vitamins, enzymes at a pH level above 7.4–7.8; consistent dissolution (demineralization) and the development (remineralization) of hydroxyapatite crystals due to their capacity for ion exchange, and the tooth enamel proteins capacity to develop stable chemical bonds with hydroxyapatite; transfer of mineral substances through the enamel in two parallel ways – from the blood through the pulp and dentin, and backwards – from the oral fluid to the tooth enamel; the development of difference between the hydrostatic (osmotic) pressure between blood, pulp tissue fluid, dentinal, enamel and the oral fluid, as well as the thermodynamic effects (temperature drops), electroosmotic phenomena caused by electrokinetic activities at the liquid phase – solid phase border [10-14].

Enhanced remineralization with further suspension (reduction) of carious lesions progress could be achieved through early detection of initial caries in the child population while there are still intact surface layer and the organic enamel matrix, which is the nucleation zone for hydroxyapatite crystal growth, combined with the remineralization therapy. Fissure caries, which is on the top of the dental carious lesions list, occurs immediately after tooth eruption. There is a proven direct correlation existing between the risk of developing fissure caries and the initial degree of the dental enamel mineralization [15, 16].

Difficulties in diagnosing permanent teeth fissure caries after eruption are due not only to the specific anatomical configuration and architectonics of the erupting tooth's chewing surface (fissure morphology; hypomineralized areas prevailing over mineralized ones; a longer period of hypomineralization of the chewing surface compared with the tooth smooth surface; greater depth of fissures in case of hypofluorosis; accumulation of food remains; retention of microorganisms in the pit-and-fissure areas with developing aggressive dental plaque, impossible physiological mineralization of enamel; constant presence of cariogenic bacteria and digestible carbohydrates in the oral cavity), but also due to the inconsistent standards in the evaluation criteria used through morphological studies [17-19].

One of the advanced and innovation-based methods employed for studying the maxillofacial area is cone-beam computed tomography (CBCT), which allows composing 3D images. Despite its advantages compared to the other diagnostic methods (obtaining a precise, high-quality, virtual 3D model of the scanned area with further cut of axial slices; minimum radiation stress; short scan mode; compliance with the golden standard of diagnostics; minimum number of artifacts on CBC-tomograms; remote examination and registering of images in a file format; data exchange with other CAD/CAM/CAE-systems), it still does not allow sufficiently detailed imaging of the morphology and microstructures of the pit-and-fissure area axial sections for the chewing surface of the hypomineralized teeth [20-23].

Due to the static nature of the focus of this study (teeth with varying degrees of demineralization), as well as due to a lacking limit imposed on the radiation stress, a high-resolution and diagnostically accurate 3D reconstruction with potential visualization of the entire internal 3D structure of the fully preserved sample is available through microcomputed tomography (micro-CT). The contemporary national and international research and medical practice have revealed a lot of rational interest in micro-CT followed with post-processing and analysis of the obtained tomograms, whereas this interest has come not from clinical experts alone but also from the other specialists focusing on studying human and animal morphology, both normal and in case of pathology. This method (approach) has proven to be reliable in solving issues that involve reconstruction of precision biological models (anatomical structures) with further computed modeling and the study of objects implanted in biological tissues [24-28].

Speaking of the most significant advantages that microCT offers, the following should be mentioned: creation of 3D microscopic images of the sample morphology, its internal microstructure (microtexture) at submicron resolution; identification of areas with tissues that have undergone pathological alteration as well as detecting their specific features; life-time dynamic evaluation of the pathology progress and of the therapy efficiency, involving laboratory animals in the real-time mode with no need for euthanasia at any control stage through the entire experiment; in vivo studying of pharmacological dynamics; visualization of bioluminescent and biochemical changes in living cells of the experimental animals; PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) screening of samples; application, development and validation of samples and biological markers; tracking cell migration in vitro and in vivo; compliance with international legal standards and the principles of morality in humane treatment of animals [29-31].

Based on the chemical and X-ray analysis outcomes, and depending on the demineralization degree of the dental hard tissues (ascending order), experts studying caries have identified the following color-codes for the mineral density – white, light brown, brown, black spots [6, 32-34].

Despite the available published national and foreign works focusing on fissure caries in children undergoing mixed (permanent) bite, in view of the macro- and microscopic, histological, and X-ray studies, the available data concerning microCT used for diagnosing and identifying pathophysiological features of early carious lesions in permanent molars still remain scarce and unsystematic, which has been the reason for conducting this study.

Aim of study – to evaluate the potential use of microCT in improving the diagnostics of early fissure caries affecting permanent molars in children undergoing teeth eruption.

MATERIALS AND METHODS

Prior to carrying out the research involving children, a conclusion was obtained from the Committee on Bioethics as well as an expressed voluntary consent from the parents (custodians). The results of the ethical review confirmed the research protocols compliance with the national and international regulations – the World Medical Association Declaration of Helsinki, 1964 ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS, as amended by the WMA LXIV General Assembly (2013); Cl. 24 of the Russian Federation Constitution; Rules of Clinical Practice in the Russian Federation (Decree 266 issued by the Ministry of Healthcare of the Russian Federation on June 19, 2003); ethical standards by the Committee on Experiments, Standards for Clinical Trials (GOST R 52379-2005); Federal Law of the Russian Federation 323-FL ON THE PRINCIPLES OF THE PROTECTION OF CITIZENS' HEALTH IN THE RUSSIAN FEDERATION (of 11/21/2011).

The teeth structure was studied with the high-resolution X-Ray microtomograph Skyscan 1176 (Bruker, Kartuizersweg, Kontich, Belgium) (Fig. 1).

Fig.1. High-resolution X-ray microtomograph Skyscan 1176 (Bruker).

When running the microCT method, an X-ray unit with a small focal spot was used, whereas useful type of X-ray radiation was done. *The microCT scanning scheme was as follows:* the microfocus X-ray tube illuminated the object while the X-ray camera received its enlarged shadow projections. Given thousands of projections obtained at different angles as the object was rotating, the computer reconstructed a set of virtual sections of the object. The operator could view the slices one following another, obtained sections at any angle and the numerical features of the 3D inner microstructure throughout the entire bulk or a particular selected area under the examination. The advanced design combined a movable stand and an X-ray camera, which allowed getting high resolution, easy placement of larger samples, and an increase in the scanning speed. Compared to the devices incorporating a fixed source-detector system, variable scanning geometry in microCT helped increase significantly the speed, and retained high resolution (Fig. 2).

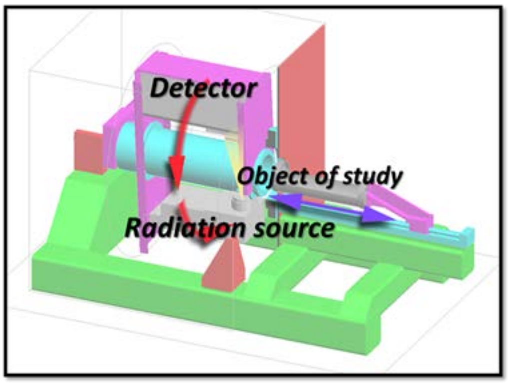


Fig. 2. Scheme of micro CT scan using an acute-focus conical beam.

The scanned objects were reconstructed with the Nrecon software (1.7.4.2, Bruker-microCT, (Kontich, Belgium)) with the following basic parameters: ring reduction 20, beam hardening 30. The spatial orientation (x, y, z) and the selection of the reconstructed areas were performed using the DataViewer software (1.5.6.2, Bruker-microCT, Kontich, Belgium) (Fig. 3).

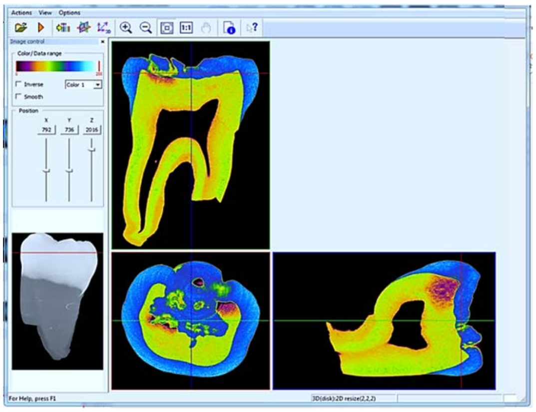
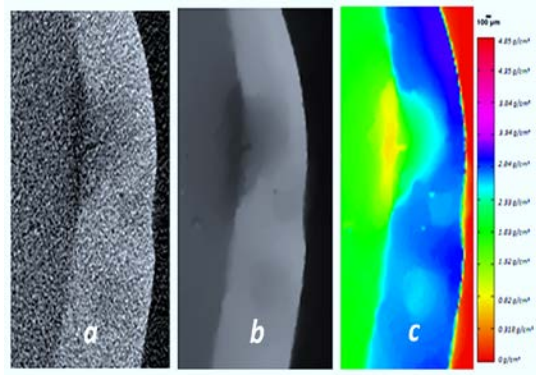


Fig. 3. Screenshot of maximum intensity 2D-projections reconstructions at the upper third of the tooth; frontal, axial and sagittal planes.

The data visualization and analysis were performed with the CT-analyzer software (1.18.4.0, Bruker-microCT, Kontich, Belgium). The 3D visualization of the obtained data, depending on the radiological density, was done in the CTvox software (3.3.0r1403, Bruker-microCT, Kontich, Belgium) (Fig. 4).



dimensional projection segmented subject to the grayscale; b - maximum intensity 2D-projection segmented according to the grayscale; c - 3D pseudo-color volumetric rendering segmented following the optical density colorimetric scale.

The tomographic study involved examining 83 first and second permanent molars of the upper and lower jaws, which were removed from children aged 8–11 years old subject to the orthodontic indications. After the extraction, the teeth were immersed in a 2% aqueous solution of Monochloramine B for 30 minutes followed with further careful removal of the remaining periodontal attachment, soft tissues, and dental (mineralized, non-mineralized) deposits. Further on, up until the microCT examination, the teeth were kept in a ready-to-use universal fixing neutral buffered 10% formalin solution (Pervaya Laboratornaya Compania, Russia). The entire bulk of the teeth was divided into two groups: the comparison group – 14 teeth featuring no signs of carious lesions and demineralization (Fig. 5a); the main group – 69 teeth manifesting carious lesions and demineralization. Depending on the hard tissues demineralization degree, the teeth in the main group were divided into the following subgroups: Subgroup 1 – 18 teeth with caries in the white spot stage (Fig. 5b); Subgroup 2 – 15 teeth with light brown spot caries (Fig. 5c); Subgroup 3 – 17 teeth with brown spot caries (Fig. 5d); Subgroup 4 – 19 teeth with black spot caries (Fig. 5e). The teeth that had combined (mixed) types of carious lesions were excluded from the study.

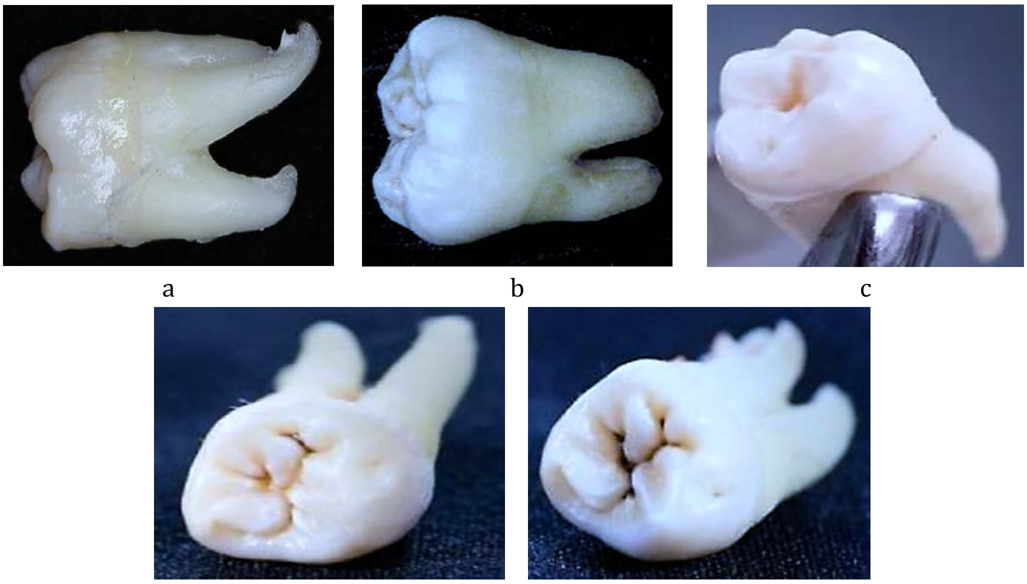


Fig. 5. Samples of teeth of the studied groups: a - without signs of carious lesions; b - with caries in the stage of white spot; c - with caries in the stage of a light brown spot; d - with caries in the stage of brown spot; e - with caries in the black spot stage.

Before determining the tooth enamel radiological (mineral) density in the studied groups with the CTvox software (3.3.0-1403, Bruker-microCT), two phantoms with already identified density (0.25 and 0.75 g/cm^3) were scanned with further determination of the attenuation rate. The obtained data was used for calibration and quantitative identification of the enamel mineralization degree in the investigated samples through micro-CT (Fig. 6).

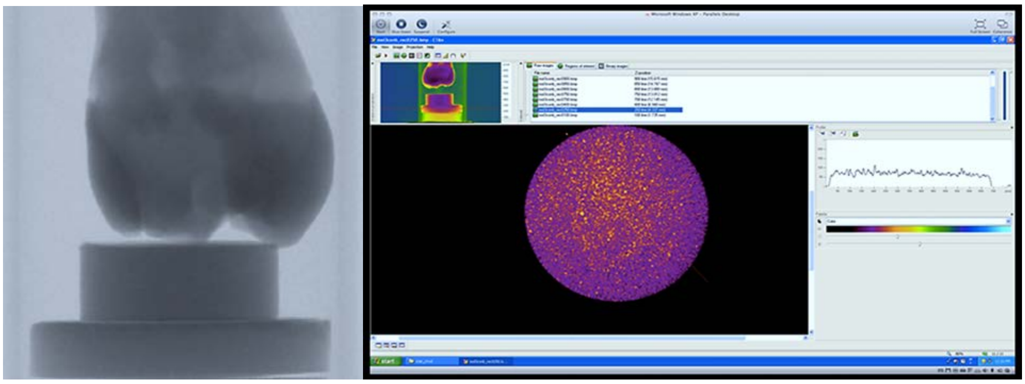


Fig. 6. Scan phantoms of a given density to determine the attenuation coefficient.

The identification of the zones (areas) for determining radiological density of the demineralized enamel was done on reconstructed 2D and 3D images by measuring the distance between the area under study and the return points, as well as in relation to the dentin-enamel junction. It is worth saying that the enamel destruction zones and enamel outer layer (50 µm) were excluded from the study. For each type of carious lesion, the demineralization degree was evaluated through the density window (diameter 0.3×0.3 mm), which directed at the studied area, and also had a one-step shift within each lesion in the mesial, distal, cheek and glossal directions. Since the enamel thickness varies significantly in the upper third of the tooth crown, the radiological density of the lesions (demineralization) was studied in the outer (0.05–0.5 mm), middle (0.75–1.25 mm) and inner (1.5–2.0 mm) thirds of the enamel layer with the average values identified, which were calculated automatically by the software, and displayed in the computer tomogram slice window (Fig. 7).

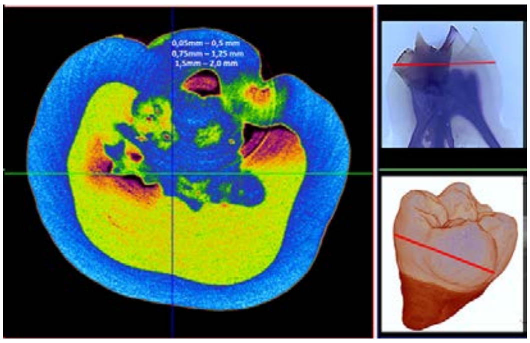


Fig. 7. Reconstructed axial slice of a demineralized molar at the upper third of the tooth crown (red line) with the examined areas marked. The side window shows the reconstruction of the examined tooth 3D images.

Statistical processing of research results was carried out using the IBM SPSS Statistics V18.0 application package at a significance level of 0.001.

RESULTS AND DISCUSSION

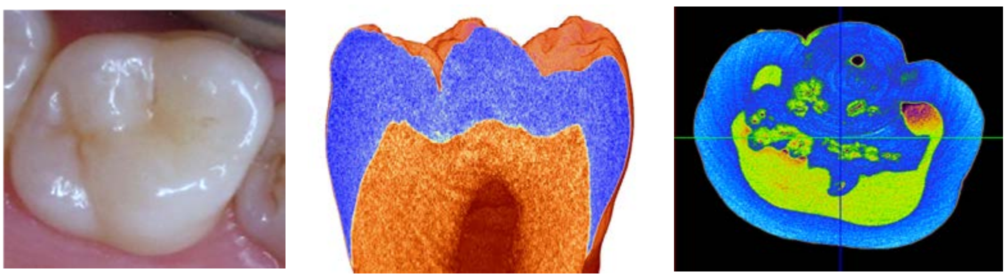
Table 1 shows the dental enamel mineral density in the groups in view of the color differentiation of the carious lesions; data obtained via microCT.

Table 1: Indicators of the mineral density of tooth enamel, taking into account the color coding of carious lesions, obtained using micro-CT, (g/cm^3), (M±m)

Carious lesion form (color coding)	Enamel mineral density study zones			
	Outer zone (thirds) of enamel thickness, 0,05-0,5 mm	Middle zone (thirds) of enamel thickness, 0,75-1,25 mm	The inner zone (thirds) of enamel thickness, 1,5-2,0 mm	Average score
Healthy enamel	2,587 ± 0,043	2,512 ± 0,064	2,356 ± 0,076	2,485 ± 0,127
Caries in the stage of white spots	2,509 ± 0,094*	2,468 ± 0,073*	2,317 ± 0,061*	2,431 ± 0,118*
Caries in the stage of light brown spots	2,375 ± 0,084*	2,358 ± 0,066*	2,263 ± 0,038*	2,332 ± 0,074*
Brown spot caries	2,167 ± 0,164*	2,181 ± 0,136*	2,199 ± 0,117*	2,182 ± 0,126*
Caries in the black spot	1,694 ± 0,139*	1,802 ± 0,141*	1,937 ± 0,216*	1,811 ± 0,117*

Note: * - statistically significant with respect to healthy enamel, (p ≤ 0,001).

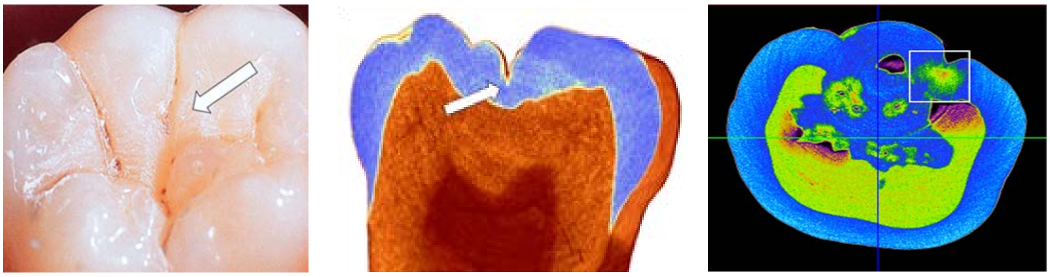
The study of the healthy (intact) tooth enamel mineral density indicated that the density was the highest in the outer zone (2.58 ± 0.04 mm), whereas the smallest density value was to be observed in the inner enamel layer (2.35 ± 0.07 mm). Many authors have offered evidence showing that the enamel surface area (as opposed to the deep layers) has been the most resistant to caries due to the significant mineralization, high physical resistance and microhardness. The gradual density increased towards the outer surface of the enamel, as was seen, was due to the following reasons. First, the diameter of the enamel basic structural units (enamel prisms) increased two-fold from the dentin-enamel borderline to the enamel surface. Second, the developed S-shaped (wave-like) bends of arcade- or hexagon-shaped enamel prism beams were found in the subsurface and central zones only. In the surface layer, the tooth enamel had no prismatic structure. The said morphological enamel arrangement prevented radial cracks that might result from the excessive chewing (occlusive) stress (Fig. 8).



a b c

Fig. 8. Photo (a), reconstructed sagittal (b) and axial (c) section of the intact molar at the level of the upper third of the tooth crown.

The evaluation of the mineral density in the early enamel carious lesions (white, light brown spots) revealed a sequence of density changes similar to that of intact teeth. The maximum density values were registered in the outer enamel zone (2.50 ± 0.09 mm and 2.37 ± 0.08 mm, respectively), the minimum – in the inner zone (2.31 ± 0.06 mm and 2.26 ± 0.03 mm, respectively) (Fig. 9, 10).



a b c

Fig. 9. Photo (a), reconstructed sagittal (b) and axial (c) section of a molar with a carious lesion in the white spot stage at the level of the upper third of the tooth crown.

a b c

Fig. 10. Photo (a), reconstructed sagittal (b) and axial (c) slice of a molar with carious lesion in the stage of a light brown spot at the level of the upper third of the tooth crown.

The gradual decrease in the density towards the dentine-enamel border was due to the demineralization center (carious lesion) having the shape of a triangle, where the base was facing the outer surface of the enamel, while the corner was aimed at the dentin-enamel junction. The following should be mentioned speaking of the factors determining the highest density values in the enamel surface (if compared with its inner layer) in case of white and light brown carious lesions. First, the number of microspaces detected through polarization microscopy at white and light brown carious spots, increased, if compared to healthy enamel (1%), reaching 5% in the affected focus of the outer layer, and 15% – in the lesion body. Second, the data obtained through the electron microscopy showed that the destruction started along enamel prisms with microscopic bonds disruption, emerging cracks, changing spatial orientation and hydroxyapatite crystals shape along with their partial destruction. The lacunae developing in the demineralization zone got filled with either organic substances or amorphous mineral salts coming from saliva. Saliva fluid in the mouth protects dental caries, erosion, scraping and periodontal diseases [35].The lacunae in the remineralization center got filled with calcium phosphate granules, while they have also been found in enamel prisms. The authors have detected no destruction in the organic stroma in the white and light brown spot stage. However, there was a disturbed connection detected between the protein matrix and mineral components in the demineralization focus. Third, micro X-ray offered enough convincing evidence proving that the mineralization degree of enamel prisms exceeded similar indicators for enamel prism membrane and the interprismatic substance, so the sequence of demineralization went on as follows: enamel prism membrane, interprismatic substance, and enamel prisms. The decrease in the content of calcium-phosphorus compounds, fluorides and other mineral components in the inter-prism substance affected the carious lesions at the white and light brown spot stage, promoted the expansion of inter-prismatic microspaces and enhanced the demineralization in the area.

Fourth, experts have noted that caries at the white and light brown stage with a lesion area not exceeding 1 mm^2, progressed with two zones developing – transparent and dark. During that, the subsurface enamel demineralization went on with no damage to the deep enamel layers and the dentine-enamel compound, while the outer enamel layer was less altered over the lesion than the deeper layers were. It has been believed that the continuous remineralization on the part of the oral fluid and the morpho-structural specifics of the enamel outer layer helped the outer enamel layer retain its relative integrity for rather a long time, as well as maintain its optical density. As it turned permeable to colorants in the white, light brown stage, the initial (early) demineralization, as well as the remineralization therapy efficiency, could be identified with caries markers (colorants) at the clinical examination.

The analysis of the enamel carious lesions mineral density in the stage of brown and black spots pointed at the inverse sequence of the density changes compared with the white and light brown color foci. The highest density was recorded in the enamel inner zone (2.19 ± 0.11 mm and 1.93 ± 0.21 mm, respectively), while it was at its minimum in the outer zone (2.16 ± 0.16 mm and 1.69 ± 0.13 mm, respectively) (Fig. 11, 12).

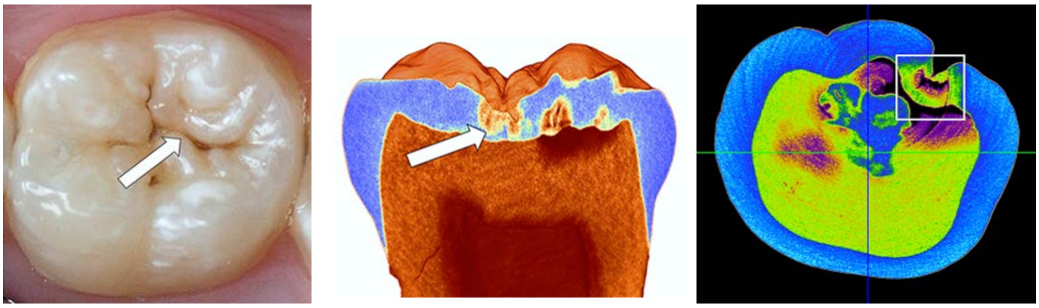


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Fig. 11. Photo (a), reconstructed sagittal (b) and axial (c) section of a molar with a carious lesion in the brown spot stage at the level of the upper third of the tooth crown.

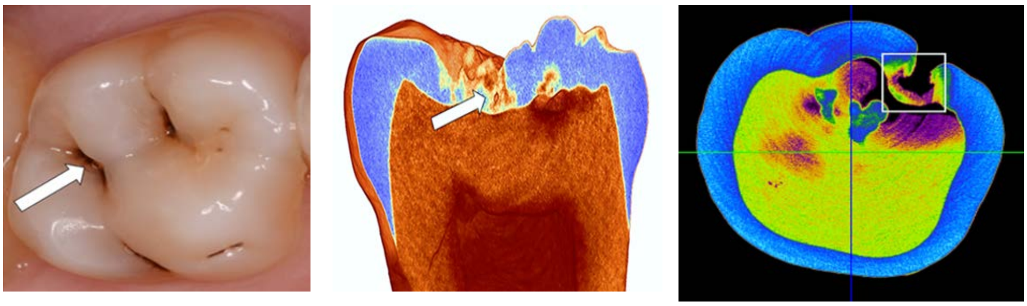

Fig. 12. Photo (a), reconstructed sagittal (b) and axial (c) section of a molar with a carious lesion in the black spot stage at the level of the upper third of the tooth crown.

The consistent density increase towards the dentin-enamel border, from this point of view, was due to the fact that the carious lesion (demineralization) focus in the cross section was trapezium-shaped where the broad base was facing the outer enamel surface, and the narrow base – the dentin-enamel junction. The following should be mentioned out of the factors that determined the highest density towards the enamel inner surface. First, the “body” of the carious lesion detected through polarization microscopy was localized in the subsurface area, and was the largest area of prominent demineralization. As the authors observed, the lesion “body” had an up to 20% decrease in the mineral substances content, an increase in the volume, as well as an up to 25% expansion of the interprismatic microspaces and a significant permeability increase along with well-visualized Retzius striae. The changes in the carious lesions "body" also affected the crystal structure, which was manifested through a violated spatial orientation of crystals in the morphological structure of hydroxyapatites, altered dimensional and volume parameters, and the appearance of crystals that were atypical of normal enamel. Besides, due to the occurring redox processes, the organic substances diffusing into the enamel turned into melanin-like substances, which colored the demineralization focus brown (black). Second, the structure of the "dark" zone located on the border with the carious lesion "body" towards the dentin-enamel compound, was described by the experts as an area with an insignificant (2–4%) volume of microspaces. This zone revealed the predominance of the remineralization over the demineralization that occurred in the lesion "body".

Third, the structure of the "transparent" zone, which was bordering on the "dark" carious lesion zone towards the dentin-enamel junction, was described by the experts as a hypermineralized zone with the least damage. The pores resulting through the removal of acid-soluble carbonate from the apatite crystalline lattice in this area were localized along the enamel prisms, while the volume of microspaces matched with the "surface" zone (0.5–1.0%). The mineralization degree in the projection of Retzius striae corresponded to the indices of healthy (intact) enamel. Therefore, the outcomes of the study clearly demonstrated the capacity that microCT with subsequent image analysis and mineral optical density evaluation can assess the alteration that the demineralization made on the tooth enamel through all the stages of caries progress.

CONCLUSIONS

1. Microcomputed tomography, as a high-tech, advanced and precision technique, allows visualizing the internal structure of the tooth as an organ in the oral cavity, expanding significantly the potential of the tools for quantitative and qualitative analysis and minimizing errors associated with the instrumental measurements. Unlike X-ray, microCT allows obtaining maximum intensity 2D-projections and 3D-reconstruction of pseudo-color volumetric rendering with minimal sample preparation. The use of 3D pseudo-color volumetric (3D) staining in microCT allows a clear and highly accurate visualization of the demineralization (carious lesion) foci in the tooth enamel differentiating them from healthy solid tissues featuring similar optical (radiological) density parameters.

2. As a non-invasive method, which also has a capacity for obtaining highly accurate data (resolution from dozens of nanometers to millimeters) within a short time, microCT has the following functions that should be considered meaningful and significant from the diagnostic point of view: the determination of optical (radiological) density; the quantitative measurement of geometric values on the reconstructed tomograms; the evaluation of linear dimensions in any plane (axial, frontal, sagittal); the reconstruction of 3D objects with further identification of their volumes.

3. Following the results obtained through microCT examination of the teeth in the above-said groups, the averaged indicators of the mineral optical enamel density for intact (healthy) teeth, as well as for teeth affected with various enamel carious lesions, were identified. The following scale (gradation) for the optical density parameters was identified (descending order): healthy enamel (2.485 ± 0.127 g/cm^3) – white spot caries (2.431 ± 0.118 g/cm^3) – light brown spot caries (2.332 ± 0.074 g/cm^3) – brown spot caries (2.182 ± 0.126 g/cm^3) – black spot caries (1.811 ± 0.117 g/cm^3). Identifying the correlations between the carious lesion (defect) color and the dental enamel mineral density (mineralization degree) expanded the understanding of the mechanisms behind caries pathogenesis, and helped improve therapeutic and preventive measures aimed at achieving a higher level of resistance to caries.

4. Microcomputed tomography, along with the other special methods (histological, stereomicroscopic, radiovisiographic, optical, etc.) suggested that fissure caries can be viewed as a consistent and gradually progressing destructive process affecting solid dental tissues (from focal demineralization to cavity development), which described the relationship between the intensity (severity) of inner issues (alterations) and the external damage (defects). The obtained data suggested the need for active interventions aimed at preventing and stabilizing demineralization in the solid dental tissues through the introduction of individual prevention programs for child population.

5. Microcomputer tomography, as a morphologically significant method for obtaining clear structural images of the solid dental tissues status, can not only complement, but also replace the histological analysis in dental studies allowing the examined object to remain intact.

6. The morphology and microanatomy of the tooth cavity and the canal-root systems in various function-oriented groups of teeth are variable and extremely complex. Microcomputed tomography, as a highly informative method of three-dimensional radiology, allowed detailed studying of all the features of the dental cavity's inner structure with an option of obtaining high-quality images of external and internal anatomical features.

7. The unification of the microcomputed tomography along with the potential reproduction of the obtained measurements and the interpretation of the results in the universal medical DICOM format allowed applying thus available values in work with a cone-beam computed tomography scanner. This, in turn, allowed not only shortening the time spent on early identification of carious and non-carious pathology in dental solid tissues, but also helped evaluate the efficiency of therapeutic and preventive measures (remineralizing therapy) within the dental treatment, in view of the available baseline data.

The authors declared no conflict of interest.

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