

of the mother and frequency of respiratory diseases in children. Most mothers tend in a situation of frustration to give extrapunitive reaction (its share of responsibility for committing the act denied or downplayed), so how would compensate for their vulnerable position. More than 2/3 of all examined mothers' low self-acceptance and a negative attitude ("I'm a bad mother", "I do not know how to educate, etc."). According to the scale prevailing feelings" most women said "negative" (fear, resentment, anger, displeasure, and anxiety). Guilt (unconscious or conscious) is associated for the majority of mothers with child's illness, however, the responsibility for recovery is vested in others: doctors, relatives, caregivers (94% mothers). As a result, between a child and mother develop inappropriate relationships that are manifested in the fact that when rejecting, infantilizing against the mother, the child becomes dependent on her, and almost always needs her attention and support. High anxiety is often diagnosed in truly ill children (72%), indicating that the disruption in parent-child relations and, in General, about the disharmonious parenting style.

Child-parent relations are characterized by great emotional significance for both child and parent. They are inherent ambivalence: the desire to "keep" next to a child and at the same time apply to child requirements, appropriate to the age, that is, as a maturing individual. Often parents (moms) tend to keep those relations at an earlier age (infancy), forgetting that a child is still a form of interaction to become inadequate. The inevitable process of growing up entails a worsening of the difficulties encountered in the interaction of child-parent and irregularities in the formation of a child.

Conclusions. This interaction of the mother and the baby continues to generate anxiety in the baby, which makes him sensitive, immature and susceptible to the formation of psychosomatic disorders. Therefore, despite the powerful medication, the effectiveness of the treatment of frequently ill children remains a challenge.

Literature

1. Boboshko I. E. Peculiarities of rehabilitation of sickly children with the type of psychosomatic Constitution. / I. E. Boboshko, M. N. Salova L. A. Zhdanov // Ref. pediatrician. – 2008. - No. 11. – S. 5-17.
2. Schneider L. B. Family psychology: textbook for universities. 2 – ed. – M: Business book, 2006 – 768c.
3. Psychology of family and a sick child: textbook: a reader / authors – compilers Dobryakov I. V., O. V. Zhdarinsky - SPb.: It, 2007 – 400 c.

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3D-RECONSTRUCTION OF THE MUCOUS MEMBRANE OF THE TRACHEA WITH THE USE OF DUAL BEAM FIB/SEM QUANTA 3D FEG

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Abstract As a result of 3D-reconstructions information about the topology of compartments of ciliated epithelial cells of the mucous membrane of the trachea of rats and the spatial geometry of the ultrastructure, intracellular structures visualized, reconstructed fine structure of submicroscopic surface epithelium of the trachea details - cilia and microvilli was obtained.

Key words: 3D-reconstruction, ciliated cells, surface epithelium of the mucous membrane of the trachea.

The characterization of tissue morphology, cellular and subcellular microstructures is performed conventionally by studying thin slices of tissue preparations. However, recently the new methods of volume microscopy and 3D-histology become increasingly important for 3D structural imaging providing more complete morphological picture by yielding spatial relations of structural elements in cellular architecture as well as tissue surfaces and interfaces.

In order to understand the processes of particle motility on epithelial surface, e.g. bacterial infection initial stages, we need clear 3D imaging of living tissue interfaces (living – nonliving as well).

The present study aims to optimize visualization technique, tissue preparation and 3D-reconstruction of trachea epithelial layers. We also applied multiscale 3D correlative microscopy methods that put high-resolution electron microscopy images and volume reconstructions in context of X-ray microtomography and light microscopy data.

Scanning electron microscopy and focused ion beam (FIB/SEM) in small dual beam systems (SDB) utilizing Slice&View procedure was applied to yield accurate 3D models of the tissue.

While reconstructing a microvolume of mouse trachea epithelia in several instances bacterial bodies were identified in close proximity or entangled with epithelial cilia. 3D imaging of external microbial organisms interacting with epithelial cilia provides insights into protective function of tracheal epithelium with regard to bacterial infection. Tissue fixation captured bacteria on different stages of epithelial penetration, allowing us to study dynamics of bacterial infection process.

The results of 3D-reconstructions can be further used for volumetric evaluation as well as computer simulation of functional processes such as fluid capillary flow, gas exchange, motility, uptake and secretion.

Aims and objectives. In this paper, the goal was set to establish a new approach using a three-dimensional spatial organization of tracheal cilia epithelium intact rats.

Materials and methods. As a method for preparing a preparation for subsequent reconstruction volume standard was used for sample preparation protocol transmission electron microscopy. The tissue sample is fixed in 2.5% glutaraldehyde, followed by 1% osmic acid solution; transferring dehydrated through a series of ethanol solutions (50%, 75%, 95%) in 100% acetone; further incubated in 50% acetone + 50% and embedded in Araldite Araldite.

Prepared by the method described above sample is fixed on the stage and placed in the electron microscope Quanta 3D FEG (FEI production company), equipped with a dual beam FIB / SEM system. The peculiarity of the device is the presence of the ion gun (ion beam focused - FIB), through which a sample may etch and deposition of the protective layer on the metal pattern. Also, the device is equipped with an electron gun, a standard scanning electron microscope (SEM). Using the electron beam is possible to obtain raster image of the sample surface.

Selecting an area of interest on a sample using first system (GIS) and ion gun sprayed protective layer 0.2 microns thick of platinum to the surface the art. Further, by using an ion beam with a current of 2.5 nA and an accelerating voltage of 30 kV is etched grooves on three sides of the area of interest, thereby isolating the block (approximately 10x10x10 mm) for the subsequent reconstruction of the volume (Figure 1). With the help of an electron beam with a current of 0.1 nA and an accelerating voltage of 3 kV, an image the front surface of the block, using the detector back reflected electrons. Next, the surface of the pits to a depth of 50 nm with a focused ion beam and then receive the image surface of the block. Repeating the procedure of scanning and bleed, get a set of slides. In this study was obtained from a set of 150 slides. Since this procedure is very time-consuming and requires a lot of time, software Auto Slice & View G3 was used to obtain a set of slides in automatic mode.

For further volumetric reconstruction microanatomical structure and subcellular structures using software package Amira ResolveRT. This software is a powerful universal tool volumetric reconstruction and modeling and has been successfully applied in various fields of science and technology, such as medicine, biology, materials science, the study of nanoscale objects. With Amira ResolveRT slide set processed to obtain three-dimensional reconstruction of the object being studied. This 3D-reconstruction can be carried out in automatic mode (upgrade volume in contrast original images) (Figure 1) and manually (marking individual structures) (Figure 2-4). The program allows the calculation of such features as the linear dimensions, volume and surface area of the structure of individual parts. Performing calculations and visualization of results are carried out directly in the programming environment Amira ResolveRT.

Results and discussion. Volume electron microscopy or scanning electron microscopy using a focused ion beam - a promising method for studying biological objects. Layered etching cross section of fixed and contrast preparation of biological tissue by focused ion beam, followed by visualization of the structure of the detector reflected electrons in FIB / SEM can adequately restore the three-dimensional structure of an object from a large series of electron-microscopic images of successive cross sections. The resulting structural information at the same time allows you to visualize complex microanatomy tissue at the subcellular level.

Conclusions. As a result of the executed 3D-reconstruction has received information about the spatial geometry of the ciliated cells of the epithelial layer of the trachea of rats visualized by intracellular structures reconstructed submicroscopic fine structure of the surface parts of the epithelium of the trachea - cilia and microvilli.

Literature

1. Fundamentals of structural histology. The spatial organization of epithelia. - Sevastyanov G.A. SPb. : Science, 2005. 375 pp.
2. Mast cells of the respiratory system and the prospects of their study (review) - Krasavina N.P, Tseluyko S.S, Dorovskich V.A Bulletin physiology and pathology of respiration. 2004. № 19. C. 74-79.
3. System morphological characteristics of bronchoalveolar lavage of patients with bronchial asthma and chronic bronchitis - Zinoviev S.V, Tseluyko S.S, Far East Medical Journal. 2001. № 2. S. 14.
4. Pogorelov M.A, Golichenkov V.A, Pogorelov V.N . // Change the volume of the oocyte and early embryo mouse when gipotonii.- BIOLOGY - THE SCIENCE OF THE XXI CENTURY: The 12th International Pushchino School-Conference for Young Scientists (Pushchino, 10 - 14 November 2008). Abstracts pp. 184-185.
5. Shklover V.J, Kazan P.R, Tseluyko S.S. // Abstracts XXIV Russian conference on electron microscopy RKEM 2012, Chernogolovka, s.511.
6. Nepomnyashchikh L.M., Lushnikova E.L., Molodykh N.A., Klinnikova M.G., Molodykh O.P. // «Ultrastructure and stereology of cardiomyocytes in the development of regenerative and plastic myocardial insufficiency during ontogeny» . Bulletin of Experimental Biology and Medicine. 2011. T. 151. № 1. S. 88-94.

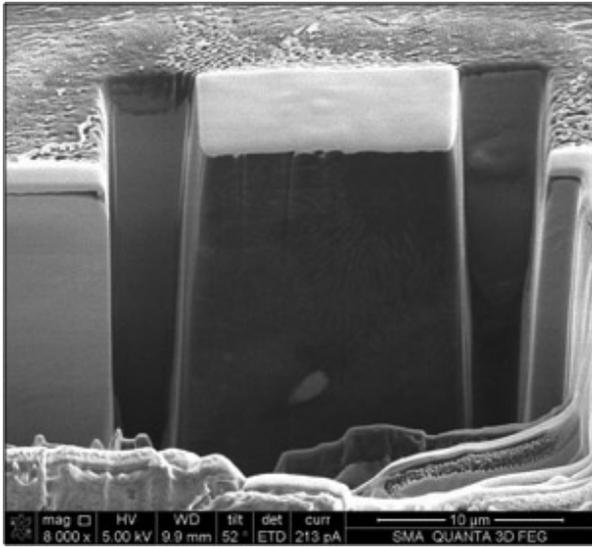
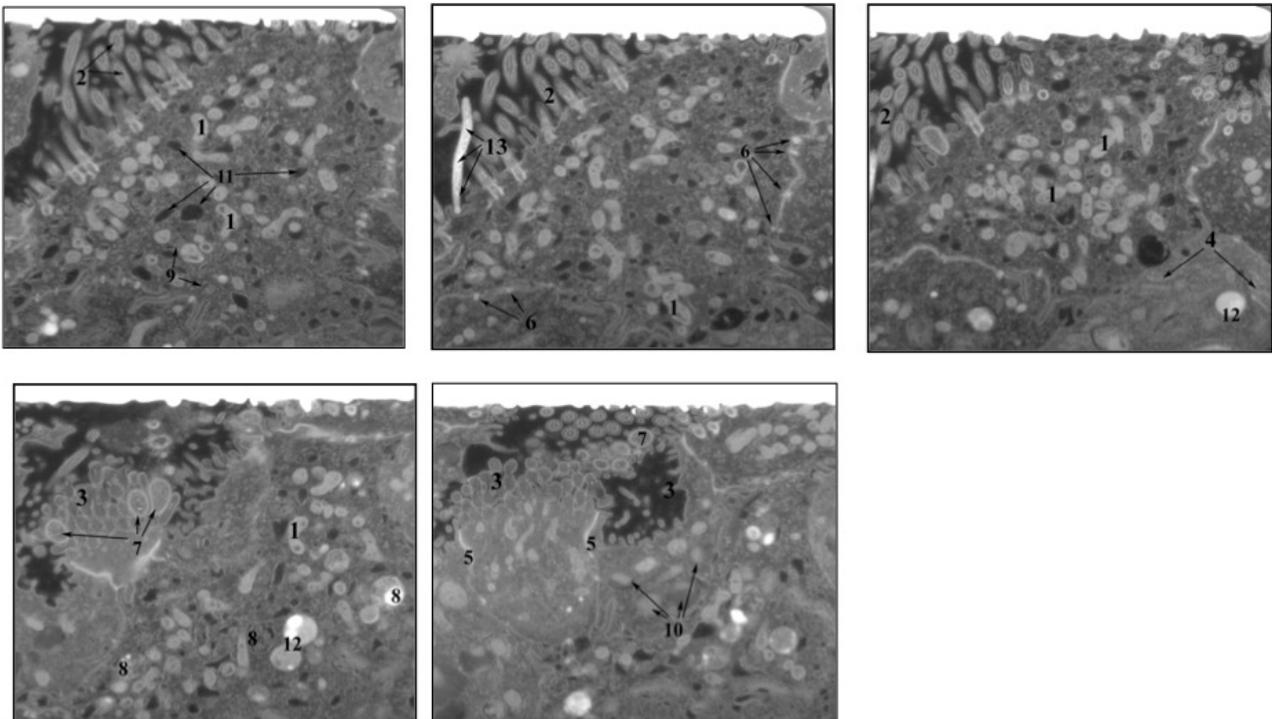


Figure 1. Block prepared to obtain a series of sections or slides. Top sputtered layer of platinum on each side etched grooves reprecipitation etched material. The intermediate step of receiving slides.



A series of successive cuts slices. 1. Mitochondria. Visible on all images, bright white structure in the case of highly branched. Inside viewed Christie. 2. Cilia. 3. Microvilli. 4, 11. The endoplasmic reticulum. 5. Cell contact. 6. Desmosomy. 7. Bacteria. 8. Organelly the heterogeneous contents. 9. The secretory granules. 10. Inclusion in the cage confining the excretory duct of protein and mucous glands of the submucosa. 12. Lizo-somy. 13. Microorganisms.

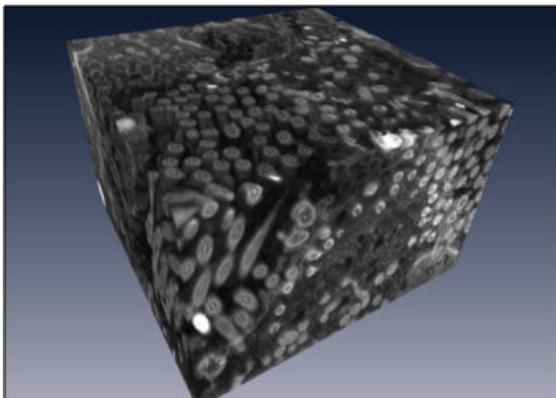


Figure 2. Reconstruction of the sample volume by the software Amira ResolveRT in automatic mode (feature Volume Rendering). The volume structure is built on the contrast of the original image.

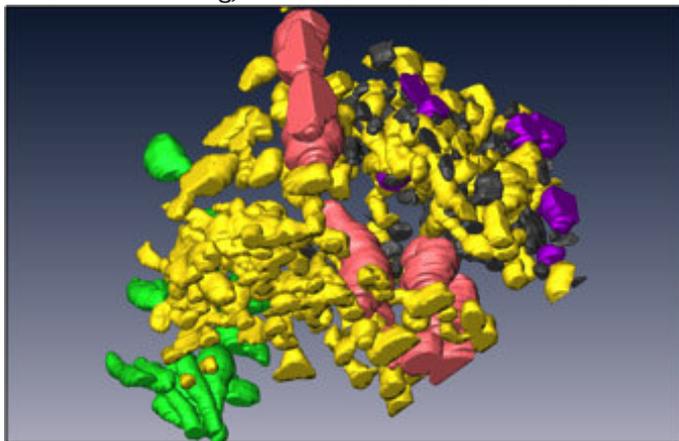


Figure 3. Reconstruction tracheal epithelium portion (a few cells). Yellow (1) marked the mitochondria, Green (2) - bacteria, pink (3) - the inclusion in the cage confining the excretory duct of protein and mucous glands, violet (4) - lysosomes, black (5) - advanced tubules EPR, green - bacteria.

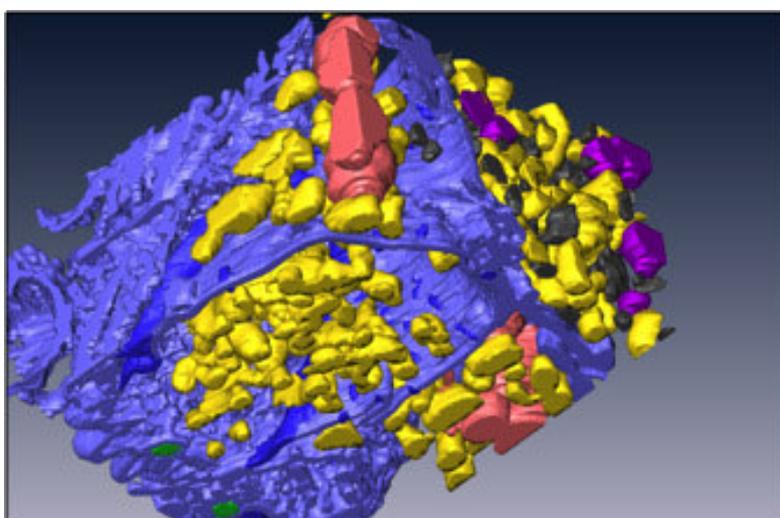


Figure 4. All the same as that in Figure 3 but with the membrane. Blue (1) denotes the membrane blue (2) - a dense cell contact and desmosomes.

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MODERN METHODS OF TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Abstract The article describes the experience in the treatment of autoimmune (idiopathic) thrombocytopenia purpura in the hematology department of the Amur regional clinical hospital. Currently available treatments for advanced high AITP, with timely diagnosis of the disease and the appointment of adequate therapy in most cases of the disease prognosis - favorable.

Key words: autoimmune idiopathic thrombocytopenic purpura, treatment.

Thrombocytopenia is a condition in which the peripheral blood platelet count drops below $150 \times 10^9 / L$. The autoimmune (idiopathic) thrombocytopenic purpura is a disease characterized by the destruction of platelets in the peripheral blood under the influence of antibodies. According to ZS Barkagan (2005), the incidence of this nosology is 4.5 males and 7.5 females per 100,000 population [1]. There are acute (lasting from 3 to 6 months) is more common in children and chronic forms of autoimmune (idiopathic) thrombocytopenic purpura (AITP) is observed more often in adults [4]. The disease develops often, for no apparent connection with any previous disease. The main clinical symptom is haemorrhage. Severity of hemorrhagic syndrome varies, from individual small bruises and petechiae to massive bleeding from internal organs and bleeding in vital organs and centers. Spontaneous hemorrhagic syndrome in these patients developed platelet counts in