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**Morphological changes of cornea after
intrastromal implantation amnion with endothelial-
epithelial dystrophy of the cornea in the experiment.
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Summary

Present the results of the morphological changes in the cornea by intrastromal implantation of amnion in an experimental model of endothelial epithelial corneal dystrophy in the rabbit.

Key words: cornea, keratopathy, amnion.

Introduction. Treatment of secondary epithelial- endothelial dystrophy (EED) of the cornea continues to be one of the most difficult problems in modern ophthalmology. The disease is consequence of inflammation, injury of the eyeball. Most of group of secondary dystrophy form postoperative dystrophy. Trigger the development of the pathology of the cornea is damaged it endothelial layer. In damaged cells is disturbed production of cytokines responsible for collagenogenesis that leads to a progressively increasing hydration of the stroma corneal degeneration keratotsitov, detachment of the epithelium and the appearance of corneal syndrome. Last circumstance makes the painful lives of patients due to permanent and no pain in the eye removed, blepharospasm, and lacrimation. [3] According to some authors, frequency of EED, as a complication after ophthalmic interventions in general ranges from 0.6 to 13% [4]. In Therefore, the problem of prevention and treatment EDD is an extremely urgent.

All existing methods of treatment are divided into EED conservative and surgical [3]. Conservative therapy, despite the use of modern drugs, physical therapy exposure (helium-neon laser stimulation, magnet), gives a temporary effect, as no eliminates the cause of pathological transformation of corneal [3,4,5]. Today the clinic is widely used selective replacement abnormal areas of the cornea, with prompted many modifications penetrating keratoplasty: mushroom, kriokeratoplastika, speed keratoplasty, intrastromal implantation of the implants polymers, Descemet membrane transplantation, etc.[3,4,5,6,7]. In recent years increasingly become the treatment of EED use of amniotic membrane. Some authors have offer to cover the surface of the cornea to protect the epithelium and its speedy recovery. Other implanted amnion under the conjunctiva to suppress excessive inflammatory response in the treatment of EED [1,2,5,6,7].

As is known, the mechanism of therapeutic action is based amnion on improving and maintaining the normal epithelialization epithelial morphotype, inhibiting the formation of coarse scar tissue. The advantage of the amniotic membrane is its biological inertness of antigenic

[1,2,6,7]. In this regard, is quite justified the use of intrastromal implantation of amnion treatment stages dalekozashedshih keratopathy.

The aim of this work was to study the morphological changes in the cornea after implantation intrastromalnoy amnion with endothelial-epithelial dystrophy cornea in the experiment.

Research objectives:

1. Studies on the effect of amniotic membrane structural elements of the cornea with a simulated endothelial-epithelial dystrophy in the experiment.
2. Conduct a quantitative assessment of morphological changes in the cornea and amniotic membrane in its intrastromal implantation model of endothelial-epithelial corneal dystrophy.

Materials and methods

In carrying out this phase of the study observed all norms of humane treatment of experimental animals, the conditions of detention and to work with them, USSR Ministry of Health established the Order № 755 of 12.08.1977, and European Convention ETS №-123 (Strasbourg, 18/03/1986year). Appraised Ethics Committee State Educational Institution Amur State Medical Academy, obtained permission to conduct this work.

The experimental part is based on the results study 20 rabbits (40 eyes), chinchilla weighing 2.5-3.5 kg at the age of 6 months. Rabbits were divided into 4 groups. The first group, control, included five rabbits (10 eyes) with simulated EED, fence the eye was performed 7 days after the start of the experiment. The second group, were simulated with the rabbits and the EED intrastromal implanted amnion 5 rabbits (10eyes), a fence eyes held 1 week afterimplantation of amnion. The third group, made up of rabbits EED and simulated implanted intrastromal amnion 5 rabbits (10 eyes), eyes held the fence 4 weeks after implantation of amnion. In the fourth group consisted of five rabbits (10 eyes) with simulated EED and intrastromal implanted amnion, fence eyes performed 12 weeks after implantation of amnion.

Stage 1. The 40 eyes of experimental animalsperformed simulations endothelial-epithelial corneal dystrophy. The method proposed in the 1971 staff Moscow Scientific Research Institutemicrosurgery of the eye and lies in the introduction of the solution 0.2-0.5% of sodium fluoride into the anterior chamber.

(Inventor's Certificate SU 1463284). When viewed through the 24 hours in animals occurred blepharospasm, lacrimation, edema of all layers of the cornea. These changes are persisted throughout the observation period.

Stage 2. After 10 days of 30 eyes after the simulation EED intrastromal implantation was performed amniotic membrane. The operation was performed under intravenous anesthesia (10% hexenal at the rate of 10-15 mg / kg body weight). Eyelids blefarostatom fixed the eyeball- fixation with tweezers, grasping limbal conjunctiva. Under control of the operating microscope, using disposable tools, the first step is peeling and removal of the corneal epithelium changed. Then at the top half of the corneal incision is carried to the rear

boundary plate along the limb, 1-2 mm from it. Then
foliate the corneal stroma within the area disc implanted
amnion. Pre-implant treated with an antibiotic solution - gentamicin and
forceps for the implantation of an intraocular lens is wound up in the
corneal pocket. Location implant permanently corrected with a
spatula. The edges of the corneal wound adapted double-
row suture 10-0. After operation for 14 days in the conjunctival cavity 6
times a day instilled a solution of ciprofloxacin 0.3%, and the solution
diclofenac 0.1%.

The operation used by native amniotic membrane person.

All rabbits was carried out post-operative examination

with a hand slit lamp HEINE HSL 150 (Germany)

with photographic recording. Withdrawal from the animal experiment

conducted under the rules set out in Annex

Number 4, "The order of euthanasia," Order of the Ministry of Health of the USSR № 755

on 12.08.1977year. In a large ear vein was injected the airat the
rate of 3 ml³ of 1 kg rabbit.

Enucleated eyes were fixed in 10% neutral formalin. Serial sections were
stained with hematoxylin and eosin, Van Gieson, toluidine blue. Painted
drugs were studied in fotomikroskope (Opton, Germany) with
increasing×100. The data obtained were treated by computed
morphometry.

Morphological studies were conducted at the Department of

Pathology Course of Forensic Medicine Amur State Medical Academy.

Results and discussion.

Table 1 shows the number average quantitative assessment of
morphological changes of the cornea with intrastromal implantation
of amniotic membrane.

Table number 1 Quantitative evaluation of morphological
changes in the cornea during implantation of intrastromal
amnion.

Histological elements	1 group (control) (10 eyes)	2 Group 1 Week (10 eyes)	3Group 4Week (10 eyes))	4Group 12Week (10 eyes)
The cells of the corneal epithelium with signs of degeneration	20±1,13	20±4,65*	1,5±0,28*	0,7±0,005*
The thickness of the surface epithelium (mkm.)	33,06±0,75	33,3±1,59*	30±2,1*	25,6±2,5*
Keratotsity	245±6,29	248±25,23*	15±1,27*	13±1,32*
Tissue stroma of the gap (mkm. ²)	1372±150,1	1190±180,5*	465±159,3*	195±44,1*
The thickness of the		71,9±6,02	82,3±6,01	63±4,62

amnion (mkm.)				
The thickness of the cornea (mkm/)	835,07± 74,07	860,3±26,62*	422,8±17,54*	390,4±43,26*

Note: *---P <0,05 - reliability of differences in relative to the control group.

For morphological study of corneas in the control group observed changes characteristic of endothelial epithelial dystrophy.

Epithelium is stratified, in able balloon dystrophy. The number of cells able to dystrophy, $20 \pm 1,13$ m on 100000^2 . The boundary between epithelium and stroma clearly traced. Stroma presented hydrated collagen fibers, among which there are a large number of cavities (Tissue cracks), occupying an area of $1372 \pm 150,1$ m² on

100 000 mm². Descemet's membrane consists of a dense network

arranged thin collagen fibers. Endothelium represented by a single layer

of flattened cells. Thickness Corneal $835,07 \pm 74,07$ m. In the observation of the second group received the following data: during the first 7 days after implantation of intrastromal amniotic membrane in experimental animals died down phenomenon corneal syndrome: decreased blepharospasm, lacrimation, remained perikornealnaya injection significantly reduced the severity of corneal edema for 2-3 days began epithelialization. By 7 days in all rabbits occurred complete epithelialization of the cornea.

Amnion clearly contoured in the stroma of the cornea.

Histological examination of drugs, 10 eyes, showed that by this time epithelialization of the cornea over. Stratified epithelium is able to balloon

dystrophy, the cells with hyperchromic nuclei, cytoplasm

moderately vacuolated. The number of cells with signs

dystrophy, $20 \pm 4,65$ m on 100000^2 , with respect to the group

comparisons (p <0,05). The boundary between the epithelium and underlying

stroma indistinct. Strom represented by collagen fibers with signs of edema. Among them there a significant number of cavities of different sizes,

partially lined keratotsitami having an elongated form with

hyperchromic nuclei. The cavities occupy $1190 \pm 180,5$ m² on

100000 mm², with respect to the group

comparisons (p <0,05). Amniotic membrane is represented by

avascular stromal matrix, with a pronounced peripheral cellular response to the

presence of

modified fibroblasts, the thickness of $71,9 \pm 6,02$ mm. (Fig.1). Stroma

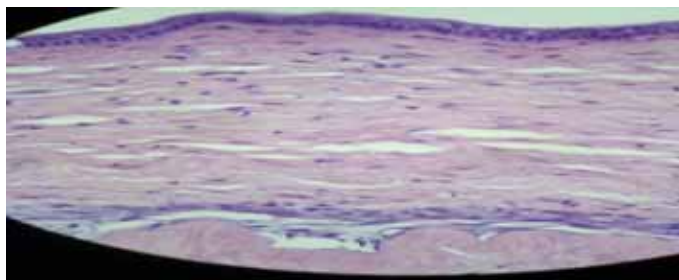
Descemet membrane closer to the represented by thick collagen fibers.

Descemets membrane consists of a dense network arranged thin

collagen fibers. Endothelium represented by a single layer of flattened cells. Thickness

Corneal $860,3 \pm 26,62$ mm, compared with the control

group (p <0,05).



In Fig. 1.

Edema of the epithelium, stroma. Cluster keratocytes on the border with amnion. 1 week after intrastromal implantation of amnion. Stained with hematoxylin and eosin. SW. $\times 100$.

In the third group there was almost complete subsided inflammation. There was no injection of an eye apple, significantly decreased corneal edema, bullous changes in the epithelium. Amnion clearly contoured in the stroma the cornea. In the study of histological preparations 10 eyes, the cornea becomes thinner, as compared to the previous period due to reduction of stromal hydration. In epithelial cells decreases vacuolization of the cytoplasm. Reduces the number of cells with phenomena balloon dystrophy $1,5 \pm 0,28 \text{ mm}^2$ to 100000 compared with the control group ($p < 0,05$). Becomes smaller cavities between the collagen fibers, reduced their size and area of $1372 \pm 150,1 \text{ m}^2$ in the control group to $465 \pm 159,3 \text{ m}^2$ ($p < 0,05$) on the 100000 mm^2 in the main, indicating that reducing the degree of hydration. Less pronounced cell reaction around the amniotic membrane. Same amnion becomes more homogeneous, there is edema, swelling and razvoloknenie stromal matrix. Its thickness increases of $82,3 \pm 6,01 \text{ mm}$. Decreases corneal thickness $422,8 \pm 17,54 \text{ mm}$ compared to the control group ($p < 0,05$) (Fig.2).



In Fig. 2. Decreased hydration of the corneal stroma. 4 weeks after intrastromal implantation of amnion. Color hematoxylin and eosin. SW. $\times 100$.

In the fourth group there were no effects of the inflammatory reaction of the eyeball. Cornea shiny, smooth, continues to increase its transparency. Amnion is not clearly contoured in the stroma of the cornea due to its

razvolokneniya. In the study drug, 10 eyes, significantly decreased the number of cells with signs balloon degeneration compared with the control group with $20 \pm 1,13$ to $0,7 \pm 0,005$ per $100,000 \text{ mm}^2$ ($p < 0,05$).

Collagen fibers are tightly prilezhat to each other that shows a significant decrease in excess hydration of the corneal stroma. Amnion is reduced in volume, compacted, tightly adherent to the surrounding material. There is no phenomenon of cellular reaction around it. His thickness is $63 \pm 4,62 \text{ mm}$. There were isolated keratotsity in the stromal matrix of the amnion. Thickness cornea was statistically decreased in Compared with the control group up to $390,4 \pm 43,26 \text{ mm}$ ($p < 0,05$). (Fig.3). After 3 months of the dynamics in the state of the cornea and amnion was not observed.



Figure3 significantly decreased hydration of the stroma corneal keratotsity evenly distributed in the stroma, there are single cells (keratotsity) in the amnion. 12 weeks after implantation of intrastromal amnion. Stained with hematoxylin and eosin. SW. $\times 100$.

On the basis of studies have indicated good survival rate of the amnion. Morphologically this is manifested active cellular response involving the region graft cells fibroplasticheskogo series - keratotsitov. In the future, there is a reduction of cell infiltration, stromal hydration and recovery the surface epithelium. Transplant itself is completely integrated into the stromal elements of the cornea and it becomes difficult to differentiate. In connection tissue is not observed any structural changes characteristic of tissue subjected to rejection or hypoxic influence. The number of transplant connective tissue fibers is different from the stroma the cornea. When stained with toluidine blue only isolated areas showing signs of metachromasia, which shows degenerative changes, but rather on the mucoid swelling - fully reversible stage in the amnion. Data changes are minor and completely eliminated from the time. Feature of the inflammatory response, we associate weak antigenic properties of amniotic membrane placed in the new "environment".

Findings

1. The data obtained from histological study, evidence of successful integration amniotic membrane in corneal stroma, the reductioninflammatory

reaction, hydration of the stroma, the acceleration epithelialization of the cornea with intrastromal implantation amnion.
2. Amniotic membrane is subjected to transformation in the translucent connective tissue.

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