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RESULTS OF EXPERIMENTAL APPROBATION OF THE ANTERIOR NON-PENETRATING LAYER-WISE INVERT KERATOPLASTY

Oganesyan O.G., Gundorova R.A., Kim A.E.

The Helmholtz Moscow Research Institute of Eye Diseases, Moscow, Russia

Abstract

Nowadays the effectiveness of conservative and surgical treatment of patients with xerogenic and neurotrophic corneal ulcers is rather low.

The regional pathological process tends to relapse thus frequently bringing forth perforation of cornea with the subsequent functional and anatomic death of an eye.

The scientifically sound methodic approach for implementation of non-penetrating layer-wise invert keratoplasty was approbated under experimental conditions in 20 Chinchilla rabbits. The essence of the matter is fixation of a layer-wise donor transplantate of posterior corneal layers in the formed recipient's corneal bed.

Keywords: *layer-wise keratoplasty, experiment, Chinchilla rabbits.*

Among the causes of blindness and visual impairment injuries and diseases of the cornea take the leading place. The problem of sterile or aseptic disturbances was considered earlier [*Gundorova R. et al., 1983*].

To etiological factors of sterile ulcers belong: "dry eye" syndrome, neurotrophic keratopathy (e.g., as a result of herpetic infection); keratopathy due to incomplete closure of lids (in case of the VII pair of cerebral nerves paralysis); in case of autoimmune diseases (for example in case of rheumatoid arthritis, Sjögren's syndrome), primary or secondary dystrophy of the cornea. Patients with xerogenic and neurotrophic ulser of cornea deserve a special attention, as the efficiency of conservative and surgical treatment is low [*Brzheskij V., Somov E., 2002*].

The recurrent erosion of cornea observed in case of diseases and pathology states considered by us frequently bring forth perforation with the subsequent functional and anatomical death of an eye. According to scientific publications the frequency of such complications makes from 10 to 90%.

As a rule, in case of mentioned complications patients are recommended to undergo layer-wise or perforating keratoplasty aimed at tectonic outcomes [*Puchkovskaya N., 1971*]. Transplantation of limbal stem cells is also possible as an addition to performed manipulations. However, as a rule, the primary ethiopathogenetic factor results in xerogenic damage of the transplant and its lysis with the relapse of ulcerative process because of which the surgical interventions (repeated keratoplasty) should be done in patients anew [*Komakh Yu. et al., 1997*].

Neither penetrating, no layer-wise keratoplasty is a guarantee (essential condition) for absence of a relapse of cornea damage in this category of patients. This circumstance gave us the idea to develop a new modification of keratoplasty aimed at treatment of destructive processes of cornea with prevention of their relapses. The properties of Descemet's membrane underlie this idea. It is known that Descemet's membrane is the most stable structure of the cornea; it is of utmost resistance to mechanical and enzymatic damages.

The new modification of the anterior layer-wise keratoplasty was developed (currently the priority reference is obtained) and approbated by us under conditions of the experiment: anterior layer-wise invert keratoplasty the essence of which is fixation of layer-wise donor transplant of posterior layers of cornea, involving Descemet's membrane in the recipient's corneal bed

Address for Correspondence:
The Helmholtz Moscow Research Institute of Eye Diseases
14/19 Sadovaya–Chernogryazskaya Street, Moscow, Russia
Phone: 8 926 204 70 28
E-mail: oftalmolog@mail.ru

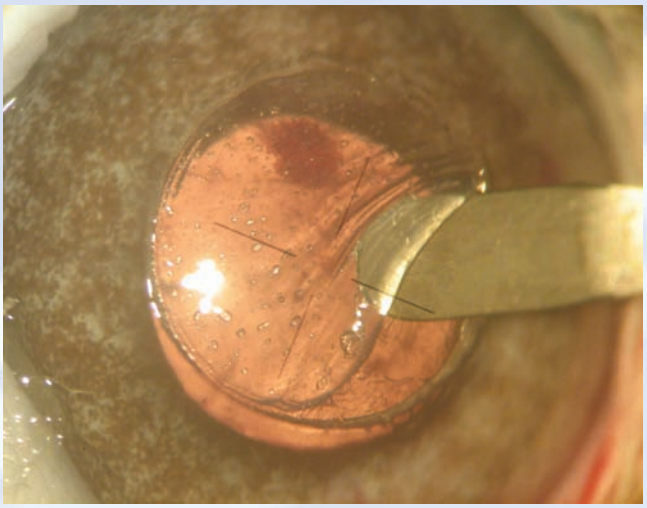


Photo 1. *Rabbit eye. Surgery stage. Tailoring the bed in recipient's cornea.*

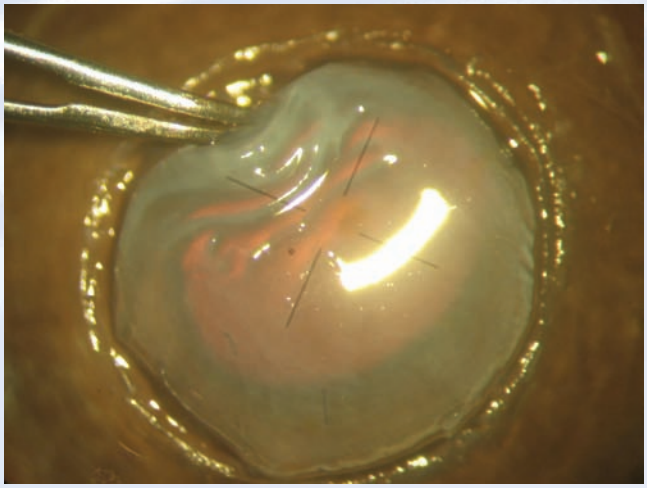


Photo 2. *Another stage of the same surgery intervention. Placing the layer-wise invert donor keratotransplantate in recipient's bed.*

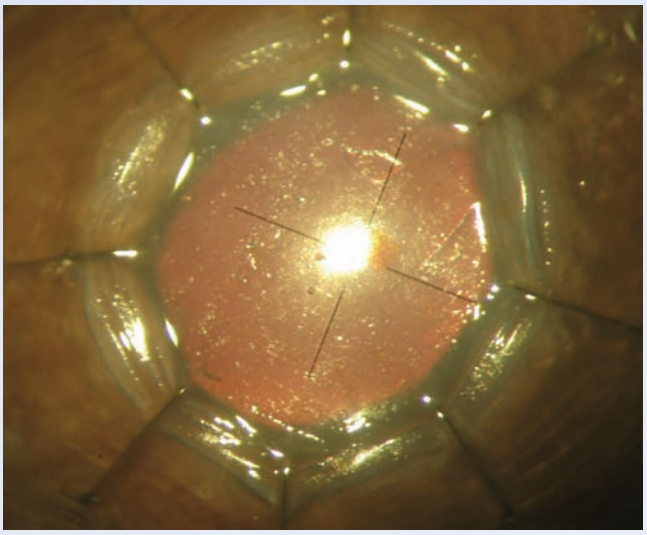


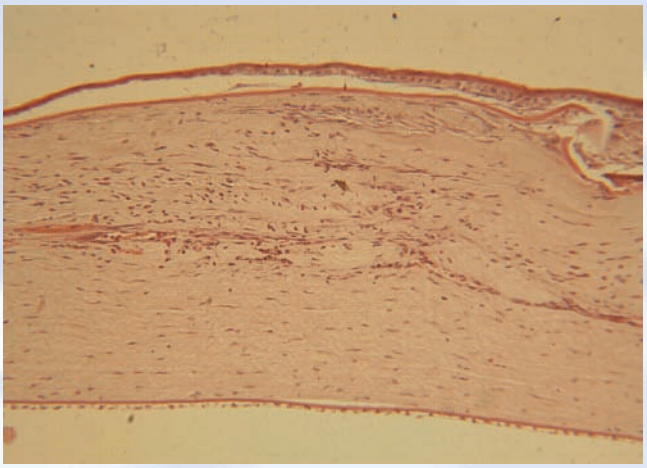
Photo 3. *Final stage of surgery intervention. Layer-wise invert donor keratotransplantate fixed by interrupted sutures.*

Photo 4. *Rabbit eye. 28 days post surgery. Transparent engraftment of layer-wise keratotransplantate.*

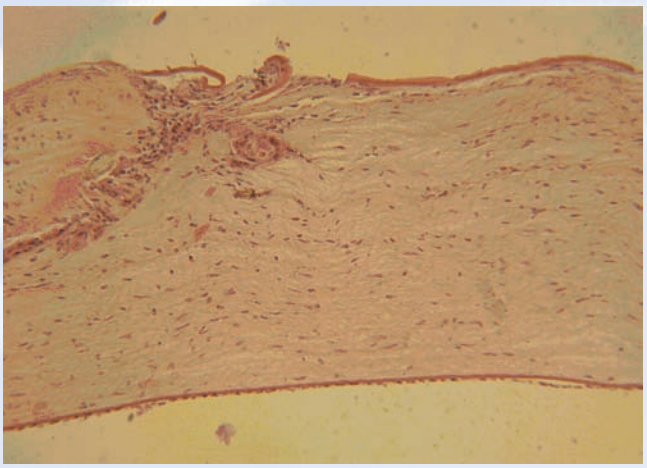
(Descemet's membrane outwards) (Photos 1-3).

The study involved 20 Chinchilla rabbits, in 40 intact eyes of which we performed the newly developed anterior layer-wise invert keratoplasty. The periods of observation varied from 1 week to 3 months (according to periods of sampling biomaterial for histological investigations), thus allowing us to study the keratotransplantate engraftment in dynamics.

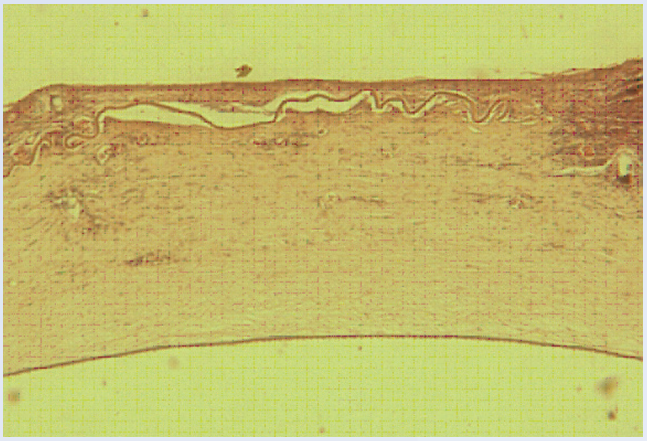
Biomicroscopy, photovideorecording, fluorescein staining were used for the unbiased assessment of engraftment. The following data was obtained upon observation: during the first 2-3 days after keratoplasty the moderate oedema of the cornea; on days 4-5 the epithelialization of the keratotransplantate began from the edges (periphery) and then proceeded concentrically towards the centre of the transplant (≈ 0.5-1 *mm* a day). By day 7-9 there occurred a complete epithelialization of keratotransplantate. By day 14 there was a complete abatement of inflammatory manifestations. The interrupted sutures of cornea were removed on day 14-28 depending on the rate of cicatrisation and neovascularization in the area of sutures. By day 28 transparent/clear engraftment of keratotransplantate was observed in 30 eyes (Photo 4), semi-transparent engraftment was recorded in 4 eyes and vascularized corneal leukoma was registered in 6 eyes. No dynamics was noted in the state of the cornea and keratotransplantate after 28 days



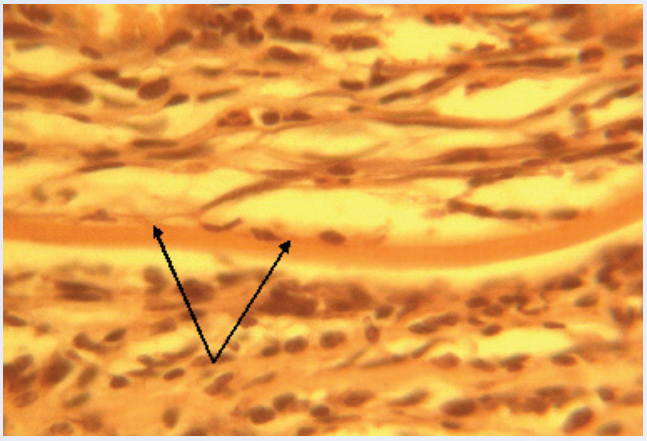
Picture 1. *Day 7 post operation. Periphery of the operative site. Increase of corneal epithelium (E) along the Descemet's membrane of a transplant. Staining: hematoxylin and eosin. × 60.*



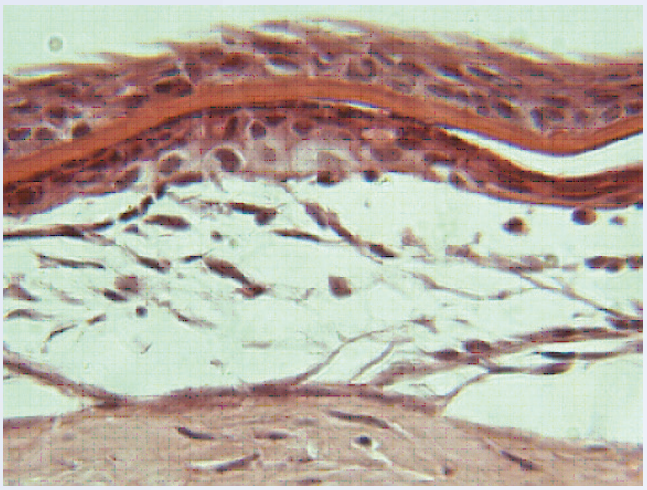
Picture 2. *Day 7 post operation. The edge of a transplant. The suture material, postoperative cicatrix, newly formed vessels, perivascular leukocyte infiltration are observed. Staining: hematoxylin and eosin. × 100.*



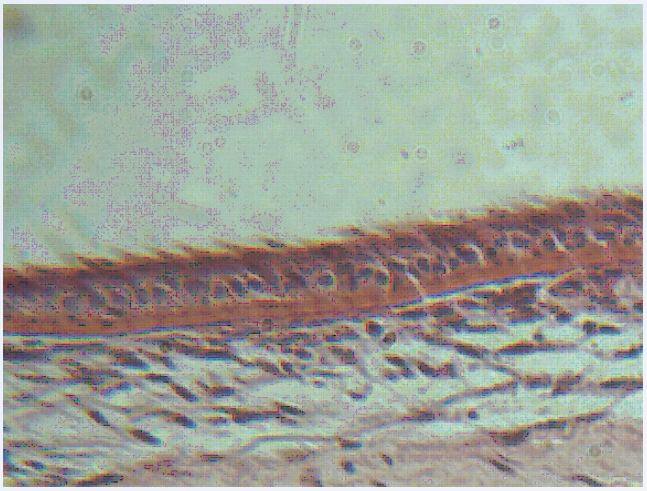
Picture 3. *Day 14 post operation. General view of cornea in the site of transplantation. Staining: hematoxylin and eosin. × 25.*



Picture 4. *Day 14 post operation. Singular endothelial cells (↑) preserved on the Descemet's membrane. ×300.*



Picture 5. *Day 28 post operation. Between the epithelium and Descemet's membrane there is a loose edematous fibrous tissue. Epithelium covering the Descemet's membrane comprises 3-4 layers. Endothelium between the epithelium and Descemet's membrane is lacking. Staining: hematoxylin and eosin. ×500*



Picture 6. *As a result of complete resolution of donor's epithelium the epithelium is all along closely adhered to Descemet's membrane. x 100.*

of observation.

The following data was obtained due to histological investigation: on day 7 after the surgery intervention the complete epithelialization of keratotransplantate was determined; however, at this period the epithelium is non-differentiated and presented by 1-2 layers of epithelial cells of prolonged elongated form (Picture 1). The oedema and leukocyte infiltration of stroma and donor's disk were recorded, as well as newly formed vessels with the maximum density in the limb area (Picture 2). Endothelial cells of the transplant failed to be traced along the entire microvessels. There was recorded a tight linkage, adhesion of the epithelial stratum to the endothelium of the transplant. Meanwhile the Descemet's membrane of a donor retained the normal structure.

By day 14 the decrease of oedema antinflammatory reaction was recorded. The epithelium was presented by 2-4 layers of low level differentiated cells (Picture 3). The endothelium of donor's disk was presented as single existing cells; no changes were observed in the Descemet's membrane of a donor (Picture 4). The thickness of cornea in the place of surgery intervention was 1.5-3 times increased compared to adjacent sites due to stromal oedema, inflammatory infiltration, as well as newly formed vessels within the transplant and underlying stroma of a recipient.

By day 28 post operation, oedema and donor's disk decreased in thickness from the initial size due to reduced oedema. The epithelium was already entirely differentiated and contained 3-4 layers (Picture 5). Hyperplasia of epithelium comprising 8-10 layers was observed at some sites, thus leveling the unevenness of the stromal surface. The basal cellular layer was differentiated all along the membrane. The epithelium tightly adhered to the Descemet's membrane of a donor as a result of complete resolution of the donor's endothelium (Picture 6). Descemet's membrane of a donor's disk preserved the normal structure. Singular minor clusters of inflammatory cells were observed only on the periphery of the transplant; this latter might be associated with the presence of sutures.

By the 3rd post-operative month no negative dynamics was observed in the state of cornea and keratotransplantate. The epithelium had a normal structure. The endothelium of a transplant entirely resolved as a result of which there was revealed a dense coalescence of the epithelium with the unchanged Descemet's membrane of a transplant along its all structural organization.

Data thus obtained allow to draw a conclusion that Descemet's membrane of a donor is capable to exist in anterior layers of cornea. Neither Descemet's membrane, nor the endothelium hinder the growth of epithelium. The obtained data allow us to launch limited clinical tests of the described methodic procedure.

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