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# The significance of fibroblasts in experimental modeling of proliferative vitreoretinopathy

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**Aim** — to investigate the role of heterogeneous fibroblasts in the development of epiretinal membrane in eyes with modeled proliferative vitreoretinopathy. **Material and methods.** The material for investigation were 6 eyes of 3 Chinchilla rabbits. Suspended fibroblasts (fibroblasts of the human skin — 200000 cells in 0.1 ml) were injected into the vitreous cavity via the pars plana. The animals were followed up for 1 month and then made out of the experiment. The eyes were enucleated and fixed in 10% neutral buffered formalin for routine histological examination. Microscopy was performed on the Leica system. **Results.** The main clinical and morphological criteria for a rabbit model of PVR induced by intravitreal injection of heterogenic fibroblasts have been established: epiretinal membrane formation, changes in intraocular structures (the retinal pigment epithelium and retina), and inflammation (due to transplantation immunity). Particularities of the epiretinal membrane development and the role of different intraocular structures have been described. **Conclusion.** The experimental fibroblastic model of PVR reproduces the final, fibrous, stage of PVR, which is significant for efficacy evaluation of antiproliferative drugs.

**Keywords:** proliferative vitreoretinopathy, fibroblasts, epiretinal membrane, retinal pigment epithelium, retina.

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Proliferative vitreoretinopathy (PVR) is a complex heterogeneous disease most often occurring as a complication after vitreoretinal surgical treatment of retinal detachment. It is characterized by proliferation of fibrous membranes which have different contractile properties and lead to traction retinal detachment [1–4].

Despite extensive research efforts, the understanding of PVR pathogenesis is still lacking. Experimental modeling has taken a leading role in studying the PVR pathogenesis. Experimental models of PVR not only helped reveal the mechanisms of pathogenesis, but also allowed assessment of the efficacy of antiproliferative drugs.

Over the course of the past 25–30 years, the main role in the development of epiretinal and subretinal membranes was found to belong to the cells of retinal pigment epithelium (RPE), glial cells, macrophages of choroid vessels — i.e. to the cells of internal eye structures [5, 6].

A prominent feature of the process of membranes' development was gradual transformation of the cellular elements into fibroblast-like cells in the result of transdifferentiation. The product of the transdifferentiation was a population of fibrous cells with active secretion of extracellular matrix that mostly consists of type I collagen. The most marked transdifferentiation was seen in RPE, where in the setting of PVR the cells went through phenotypic conversion, epithelial cells changed to fibroblast-like with a specific function: the capacity for active proliferation and secretion of extracellular matrix [7]. In this context, the most adequate experimental model to study PVR was deemed one that employed fibroblast culture. Beside faster development of epithelial fibrous membranes, such models are known to have specific ultra-

structure typical for fibroblasts that explains the appearance of contractile qualities in newly formed membranes [8]. The examination of growing fibroblasts after their implantation into vitreous body showed characteristic presence of the so-called “stress-fibrills” in their cytoplasm. Such fibroblasts with contractile function were consequently named microfibrils because they actively express a muscle protein — alpha-muscle actin. The appearance of retinal puckers and local retinal detachment was observed during the process of epiretinal membranes (ERM) development from proliferating fibroblasts confirming the contractile ability of the newly formed ERM.

Therefore, the fibroblast model reflects the outcome of proliferation observed in PVR as the result of complex immunobiological transformations of migrating cells that appear after an injury, rhegmatogenous retinal detachment and complicated inflammatory response. While the model does not include every mechanism featured in PVR for the study of its pathogenesis, it is still valuable for the effectiveness assessment of antiproliferative drugs presenting objective data on the therapeutic effect of preparations such as 5-fluorouracil, mitomycin, daunorubicin, corticosteroid [5, 9].

Additionally, morphological examination showed that activity of fibroblast cells of autologous, homo- and xenogenous (heterologous) origin are considerably important for characterization of the fibroblast model. Au-

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tologous skin fibroblasts have been studied in sufficient detail, while heterologous fibroblasts are unresearched. There have been no studies of changes in the intraocular structures in modeling by heterologous fibroblasts.

The goal of the present study is to investigate pathological changes in eye tissues of rabbits after vitreoretinal injection of heterologous fibroblasts of human skin emphasizing not only the process of ERM formation, but also the nature of reactive changes in retina and uveal tract, and their possible contribution to the proliferation process. Such analysis will enrich the understanding of the efficacy of local antiproliferative therapy.

## Material and methods

Human skin fibroblast cells were acquired from the cell culture collection of Koltzov Institute of Developmental Biology of Russian Academy of Sciences. The study material comprised 6 eyes of 3 Chinchilla rabbits weighted 2.5–3 kg. The rabbits were kept in standard conditions in vivarium at Helmholtz Research Institute of Eye Diseases. After the animals were instilled 0.5% solution of Alcaine (Alcon), they were injected 0.1 mL of cell culture intravitreally (200 000 cells in 0.1 mL of phosphate buffer) through the flat part of ciliary body into both eyes with 32G insulin syringe. The animals were then observed during 1-month follow-up employing the methods of ophthalmological examination: biomicroscopy and ophthalmoscopy. After the animals were removed from the experiment, the experimental eyes were enucleated and put into 10% formaldehyde solution and subjected to standard histological procedures. Microscopic examination was performed at 200–600 magnification using “Leica” microscopic system with integrated digital camera.

## Results

After the intravitreal injection of cell culture of human skin fibroblast into rabbit eyes, five of the six subject eyes developed PVR pattern. Morphological changes associated with PVR development in observed eyes consisted of four main aspects: 1) inflammatory process; 2) formation of epiretinal membranes; 3) changes of RPE; 4) retinal changes.

### 1. Inflammatory process

With injection of heterologous fibroblasts into vitreous body, some inflammatory changes appeared in the eye tissues. The localization of those changes had certain patterns; they mostly resided in ciliary body area and in the cup of optic nerve head (ONH). An accumulation of lymphoid cells was noted in the ciliary body in the form of round infiltrates. In the ONH cup, beside inflammatory infiltrate cells there were aggregates of fibroblast culture of various development stages including mature fibroblasts with distinctive stretched form and multiple

sprouts. The expressiveness of inflammatory infiltration in the choroid was diverse with tendency for lymphoid nodules formation. Among cell elements of inflammatory infiltrates, numerous plasmatic cells were seen; presumably, their presence reflected immunologic processes responding to antigenic action of heterologous fibroblasts.

One regular component was the appearance of transvitreal membranes in the cavity of vitreous body spreading from *pars plana* of the ciliary body to the ONH cup (Fig. 1). The membranes were noted to contain proliferating cultured fibroblasts surrounded by collagen fibers and lymphoid cells of inflammatory infiltration. Foci of necrotic detritus were also seen there. In macroscopic examination, such membranes could imitate retinal detachment.

### 2. Formation of epiretinal membranes

When fibroblast culture was injected intravitreally, they consistently spread along the inner surface of retina with ERM forming from *ora serrata* to ONH (Fig. 2). Three variants of the membranes predominated.

Variant 1 (Fig. 3). Fibrous membranes consisting of mature elongated fibroblasts surrounded by collagen fibers; those membranes caused folding of the retina.

Variant 2 (Fig. 4). Cell membranes consisting of proliferating fibroblasts with signs of starting secretion of collagen in the form of soft thin collagen fibrils. Spreading through inner surface plane of the retina, those membranes did not cause retina to fold. Internal limiting membrane (ILM) could be visualized under those membranes.

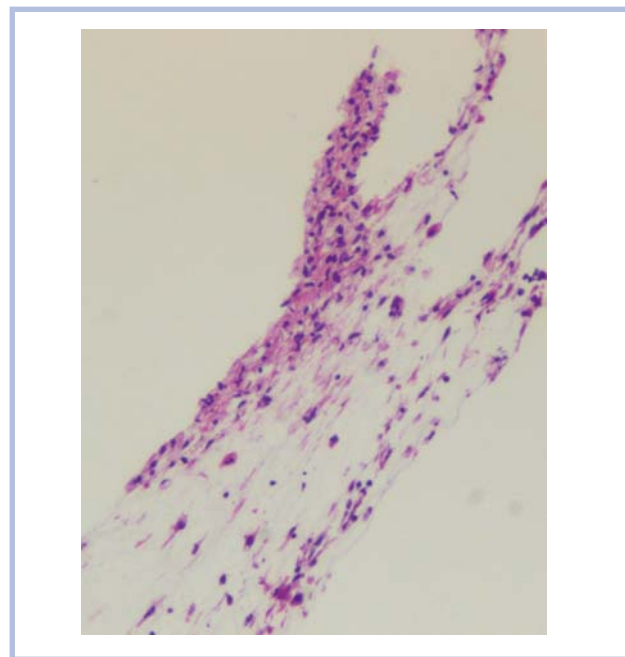
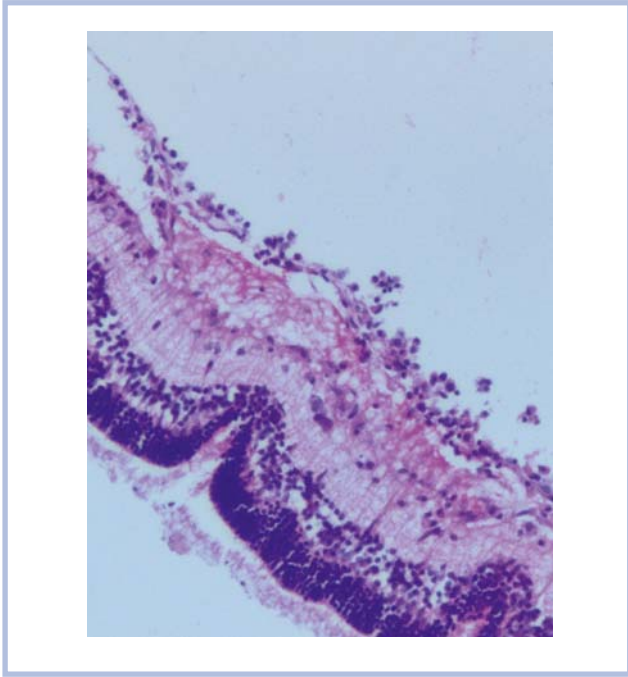


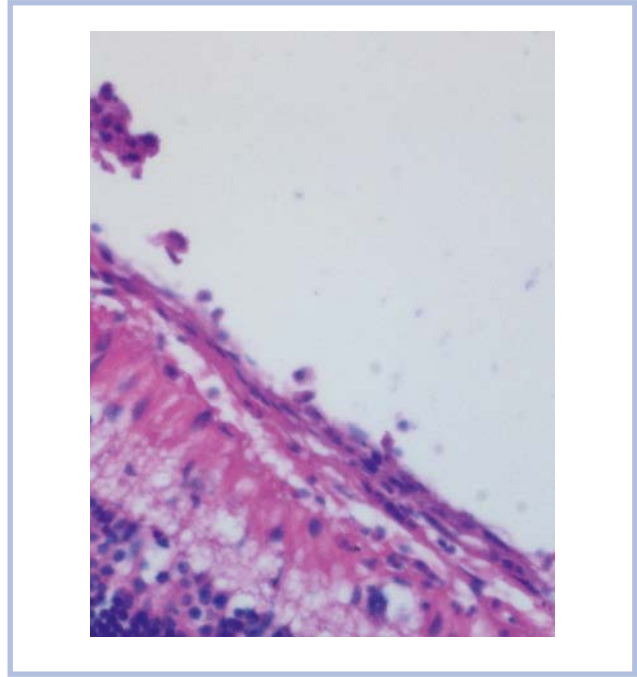
Fig. 1. Large fibrous membrane in the rabbit vitreous body imitating retinal detachment, one month after heterologous fibroblasts transplantation.

Staining with hematoxylin and eosin. 200x magnification.



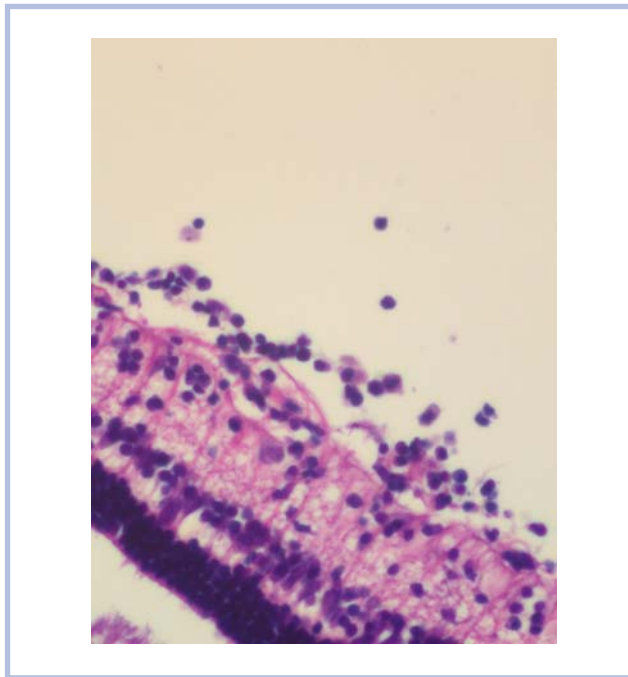
**Fig. 2. ERM after intravitreal injection of heterologous human skin fibroblasts. Overview, structural polymorphism.**

Staining with hematoxylin and eosin. 200x magnification



**Fig. 3. ERM (variant 1).**

Staining with hematoxylin and eosin. 400x magnification.



**Fig. 4. ERM (variant 2).**

Staining with hematoxylin and eosin. 200x magnification.

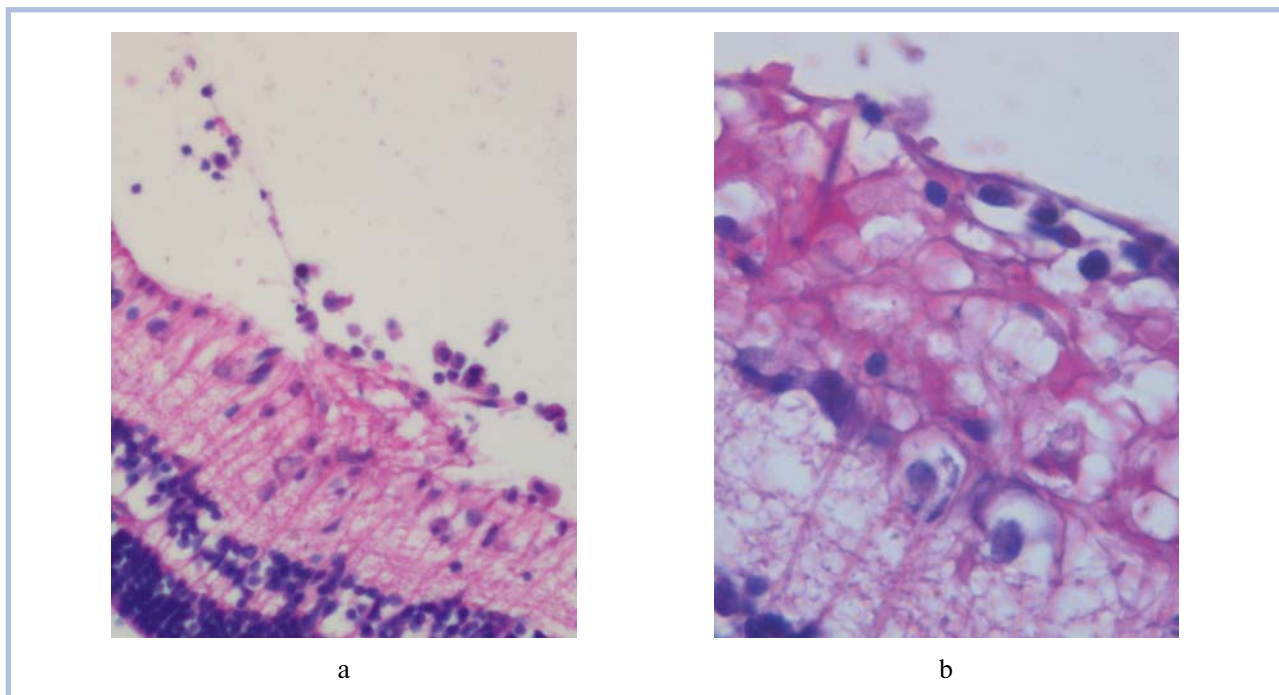
Variant 3 (**Fig. 5**). Glial membranes consisting of proliferating glial elements. Those membranes had loose fenestrated fine-fibrous structure and were closely fused with the retina. Visualization of ILM in the area of glial membranes could not be achieved. On the vitreous body

side, the membranes were covered with a thin film of proliferating fibroblasts with slim collagen fibrils.

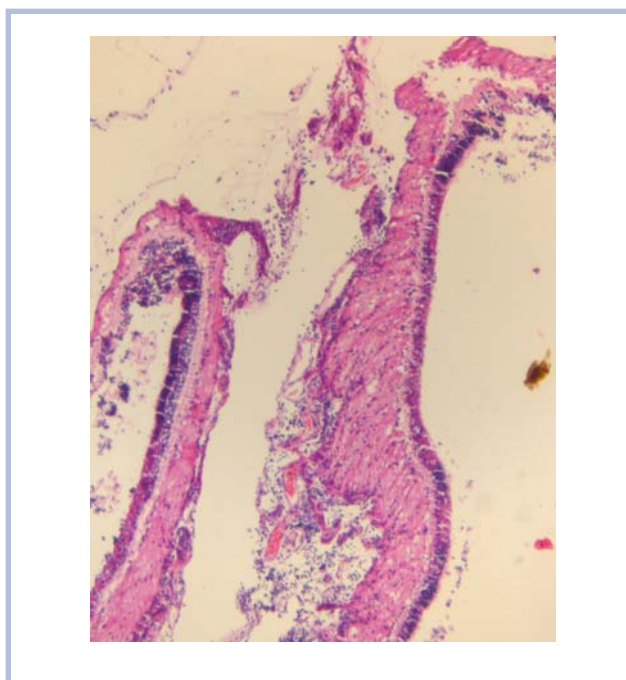
To summarize, the spreading of fibroblast culture throughout the inner surface of the retina for one month was accompanied by the formation of fibrous membranes and cellular infiltration, in which cultural fibroblasts, inflammatory cells and migrating RPE cells could be identified. It must be emphasized that along with ERM growing being horizontally oriented, traction properties of ERM were noted to contribute to the formation of retinal pucker. Traction retinal detachment in the form of a “pillar” was observed in 2 of 5 eyes (**Fig. 6**). The retinal detachment was distinctively formed in the proximity of ONH as a result of active PVR process in the central part of retina. The pathologic process resulted in the formation of funnel-shaped retinal detachment.

### 3. Pathology of retinal pigment epithelium

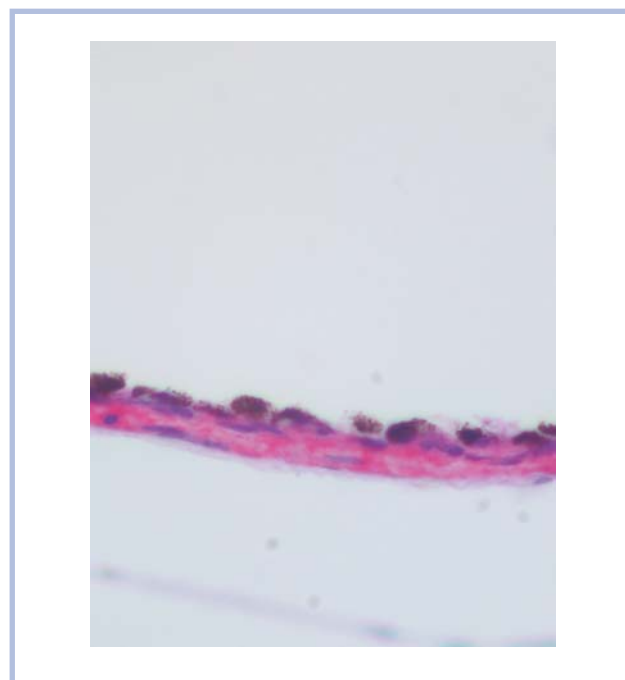
Widespread dissociation of RPE cells was observed. It involved their transformation into more round shaped cells following the loss of hexagonal form (**Fig. 7**). Disruption of adhesive properties of RPE was accompanied by the cells' migration from the sheet to the subretinal space (**Fig. 8**). The migrated cells lost pigment, and the acquisition of ability to move and a new migrational function by the RPE cells was credited for the emergence of numerous sprouts in the cytoplasmic membrane. Active migration processes resulted in the accumulation of RPE cells on the inner surface of the retina. Various transitional forms reminiscent of fibroblast-like cells with starting secretion of extracellular matrix were seen among



**Fig. 5. ERM (variant 3): growth of glial tissue (a); links between glial elements of ERM and inner retinal layer, ILM is absent (b).** Staining with hematoxylin and eosin. 200x magnification.

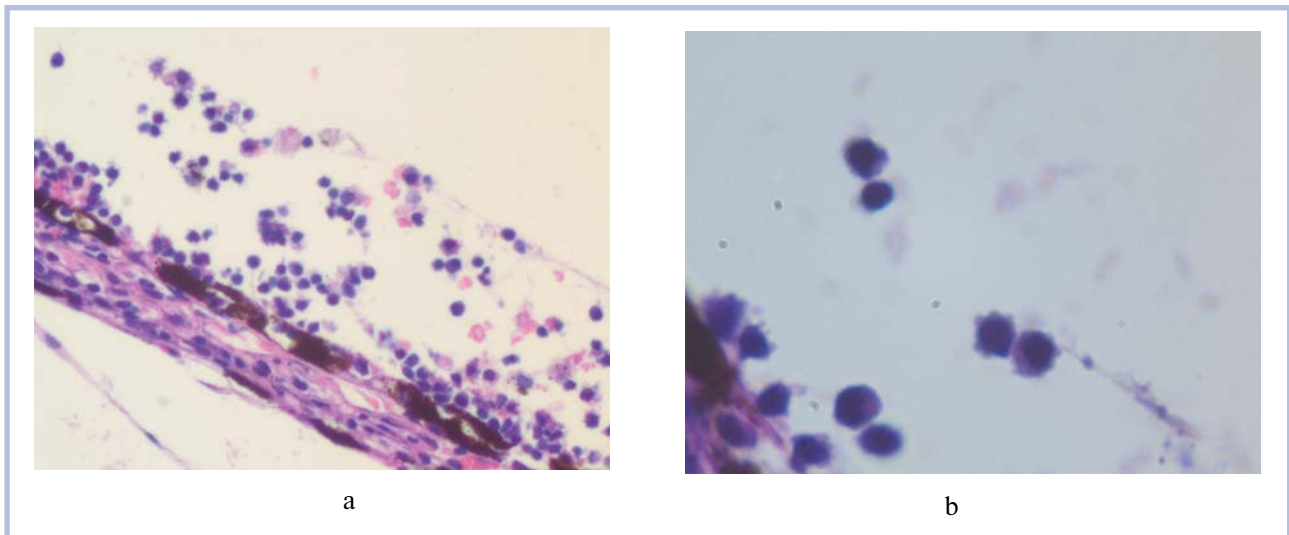


**Fig. 6. Funnel-shaped traction retinal detachment after intravitreal injection of heterologous fibroblasts (1 month).** Staining with hematoxylin and eosin. 100x magnification.



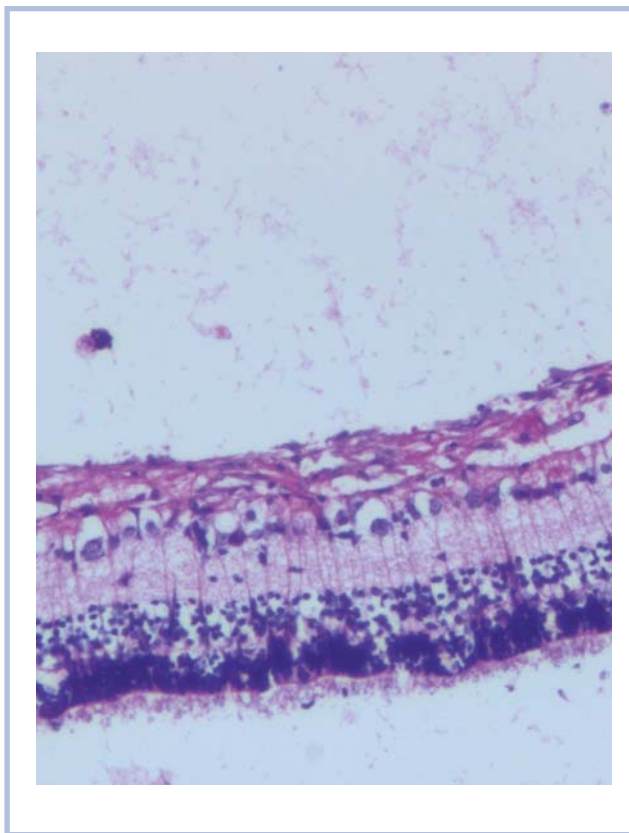
**Fig. 7. Rounding of RPE cells, break away from cell sheet.** Staining with hematoxylin and eosin. 200x magnification.





**Fig. 8. Changes in RPE: accumulation of migrating RPE cells in the subretinal space (a); migrating RPE cells with numerous sprouts, initial transdifferentiation (b).**

Staining with hematoxylin and eosin. (a) — 200x magnification, (b) — 400x magnification.



**Fig. 9. Atrophic changes in the retina in experimental PVR.**

Staining with hematoxylin and eosin. 200x magnification.

those accumulations. That can be regarded as transdifferentiation of RPE cells linked to the shift from epithelial phenotype towards mesenchymal phenotype.

#### 4. Pathology of retina

Retinal changes depended on the expressiveness of proliferative inflammatory processes accompanying the formation of ERM. Disappearance of the outer photoreceptor segments and the external plexiform layer, and thinning of the outer and inner nuclear layers were observed. The changes were more significant in the areas of retinal puckers, where the atrophic processes were more noticeable (**Fig. 9**). Such changes were attributed to the development of retinal ischemia, which presumably was linked to the pathology of RPE and its dissociation, as well as the appearance of retinal puckers and retinal detachment. The active involvement of glial elements of inner retinal layers in the formation of ERM (glial membranes) should be noted.

#### Discussion

The experimental data showed that injecting fibroblast cell culture into the vitreous cavity caused them to specifically spread along the thick base-substrate, which was the retina. Proliferating cultured fibroblasts subsided on its inner surface were modeling the ERM. This pattern of ERM distribution emphasizes the dominating role of the retina as the base substrate for ERM development

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in contrast to the vitreous body, although fibrous membrane sheets were found there as well. Three main cell subtypes reflecting the dynamics of the fibroblastic process were found in the ERM structure. Beside the horizontally oriented allocation of the cell-fiber structures, among other distinctive features of the ERM were the appearance of the retinal puckers due to contractile properties of the mature fibrous membranes, as well as traction retinal detachment. This complication is associated with contractile properties of fibroblasts — a specific contractile apparatus appeared in their cytoplasm in the form of myofilaments expressing alpha-muscle actin that can react to extrinsic stimuli [8]. The fact contradicts previous understanding of the mechanism of fibrous membrane contraction, which have been considered to be based on collagen formations [10]. Studies showed [8] that membrane contraction is based on the cellular factor together with extracellular factor exerting regulatory action on the contractile mechanisms. Thus, the ERM forming after intravitreal transplantation of fibroblasts are characterized by horizontally oriented growth and the appearance of contractile properties in the form of retinal puckers and limited traction retinal detachment.

Along with the main pathologic process linked to ERM formation, other changes of eye tissues including inflammatory response and changes in RPE and retina were observed. The inflammatory response featured elements of immune inflammation associated with heterologous fibroblasts: nodular inflammatory infiltration, plasma cell infiltration, foci of lysis. The expressiveness of the inflammatory processes was generally similar to one seen after transplantation of autoimmune fibroblasts [10].

The most expressive changes were observed in the retina. Focal proliferation of glial cells was noted on the inner retinal surface in the area of damaged ILM confirming the reparative nature of the process. This proliferation was considered a result of the action of lytic ferments in the death of incubated fibroblasts. However, the proliferation of glial cells did not involve their transdifferentiation; properties inherent to glial tissues remained despite shape change. Similar change of retinal astrocyte shape was observed in astrocytoma cell culture [11]. Coincidentally, the ability of glial cells to migrate during repair is still not well understood. The role of Muller cells in ERM formation is also a subject for discussion [12].

Active stimulation of migration processes in RPE was naturally expected after intravitreal injection of heterolo-

gous fibroblasts. The work of R. Machemer [13, 14] and further research showed the ability of migrating RPE cells to transdifferentiate causing the change of cell phenotype. Thus, beside incubated heterologous fibroblasts, the formation of experimental ERM actively involved intraocular structures: glial retinal elements (fibrous astrocytes) and migrating RPE cells. Therefore, the action of heterologous fibroblasts when injected intravitreally can be considered to be based on stimulative and inflammatory-reparative processes aimed at the formation of ERM.

## Conclusions

1. Intravitreal injection of heterologous human skin fibroblasts caused development of PVR featuring consistent horizontally oriented growth of the fibroblasts on the inner surface of retina, which served as the substrate for ERM formation. Contractile properties of the ERM conditioned the appearance of retinal puckers and limited detachment.

2. Injection of heterologous fibroblasts into the vitreous body of rabbits was accompanied by secondary reactive changes in eye tissue structures contributing to the active PVR process.

3. Endovitreous injection of heterologous fibroblasts featured:

- retinal affect: proliferation of glial elements in the process of ERM formation, as well as atrophic changes of photoreceptor and neuronal structures;

- RPE lesion: dissociation and break away from cell sheet, establishing of a pool of migrating cells with signs of transdifferentiation, involvement in the ERM formation;

- inflammatory processes in the uveal tract reflecting transplantation-immune mechanisms, and possibly promoting pathology of the retina and RPE.

4. The experimental fibroblastic model successfully reproduced the final, fibrous stage of PVR; it can be used to improve the efficacy evaluation of antiproliferative drugs.

### Author contributions:

Study conception and design: I.K-M.

Collection and handling of data: N.L., I.K-M.

Drafting of manuscript: I.K-M., N.L., E.V.

Critical revision: I.K-M., A.V.

**The authors declare that there are no conflicts of interest.**

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