MODERN ANATOMICAL AND PHYSIOLOGICAL BASES FOR MAINTAINING THE TRANSPARENCY OF THE CORNEAL STROMA. LITERATURE REVIEW

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ABSTRACT

The article presents a literature review of the modern concept of anatomical and physiological structure and functioning of the cornea. The strict morphological structure and corneal tissue homeostasis ensure its transparency. Studying the mechanisms that regulate the constancy of the corneal tissue internal environment allows us to get closer to understanding the prospects for regenerative therapy for the corneal stroma pathology. The article discusses in detail the role and functional potential of corneal stromal cells, which are capable of reverse cytologic differentiation, which primarily ensures the maintenance of tissue homeostasis and corneal transparency. The functional activity of corneal cells can change for a number of reasons, which may be exogenous, iatrogenic (trauma, infection, etc.) or endogenous. Endogenous causes include: cell autoregulation pathologies (for example, enzyme defects); defects in transport systems leading to tissue hypoxia; disorders of the neuro-humoral regulation of trophism. The physical reason for the violation of the corneal transparency is an increase in the light scattering. The article presents five main causes of increased light scattering in the opaque cornea, and also provides an overview of the main substances – components and products of cellular synthesis of corneal stromal cells: cytokines and growth factors (complex of the signal molecule and the SDF1/CXCR4 receptor, insulin-like growth factor 1, tumor necrosis factor alpha, intercellular adhesion molecule 1, erythropoietin, neurotrophic factors, etc.). Thus, corneal opacity can be caused by a single pathogenic mechanism or be the result of a complex effect of several factors. The main processes of tissue homeostasis regulation are aimed at maintaining the unique morphological structure of the cornea.

Key words: *corneal structure, keratocyte, corneal stroma, corneal transparency*

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СОВРЕМЕННЫЕ АНАТОМО-ФИЗИОЛОГИЧЕСКИЕ ОСНОВЫ ПОДДЕРЖАНИЯ ПРОЗРАЧНОСТИ СТРОМЫ РОГОВИЦЫ. ЛИТЕРАТУРНЫЙ ОБЗОР

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РЕЗЮМЕ

Статья представляет собой литературный обзор на тему современной концепции анатомо-физиологического строения и функционирования роговицы. Строгая морфологическая структура и гомеостаз ткани роговицы обеспечивают её прозрачность. Изучение механизмов, регулирующих постоянство внутренней среды ткани роговицы, позволяет приблизиться к пониманию перспектив регенеративной терапии патологии стромы роговицы. В статье подробно рассматриваются роль и функциональный потенциал стромальных клеток роговицы, которые способны к обратной цитодифференцировке, что в первую очередь обеспечивает поддержание гомеостаза ткани и прозрачности роговицы. Функциональная активность клеток роговицы может изменяться по ряду причин, которые могут носить характер экзогенных, ятрогенных (травма, инфекции и др.) либо быть эндогенными. К эндогенным причинам относят: патологии ауторегуляции клеток (например, ферментопатии); дефекты транспортных систем, приводящих к гипоксии тканей; расстройства нервно-гуморальной регуляции трофики. Физическая причина нарушения прозрачности роговицы заключается в увеличении рассеивания света. В статье приводится пять основных причин повышенного светорассеяния в непрозрачной роговице, а также представлен обзор основных веществ – компонентов и продуктов клеточного синтеза стромальных клеток роговицы: цитокинов и факторов роста (комплекс из сигнальной молекулы и рецептора SDF1/CXCR4, инсулиноподобный фактор роста 1, фактор некроза опухоли альфа, молекула межклеточной адгезии 1, эритропоэтин, нейротрофические факторы, и др). Таким образом, помутнение роговицы может вызываться как одним патогенетическим механизмом, так и комплексным воздействием нескольких факторов. Основные процессы регуляции тканевого гомеостаза направлены на поддержание уникальной морфологической структуры роговицы.

Ключевые слова: *гомеостаз роговицы, кератоциты, строма роговицы, прозрачность роговицы*

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SECTION 1. RELEVANCE OF THE TOPIC. MODERN ANATOMICAL ASPECTS OF THE CORNEA STRUCTURE. STROMAL KERATOCYTES AND THEIR ROLE IN MAINTAINING CORNEAL TRANSPARENCY

Visual impairment due to pathological changes in the cornea is the third leading problem in ophthalmology after cataracts and glaucoma [1]. The cornea is a unique refractive vascular-free, transparent and tightly innervated connective tissue structure. Its role is to provide light transmission, protect the structures of the anterior chamber of the eye and provide 2/3 of the total refractive power of the eye. The strict morphological structure and corneal tissue homeostasis ensure its transparency [2]. 90 % of the total volume of the cornea belongs to the stromal layer, scarring (fibrosis) of which leads to blindness. According to a significant statistical study, 12.7 million people worldwide are on the waiting list for a donor cornea transplant [3].

A healthy human cornea has a central thickness of 470–620 μm, the thickness at the periphery is 650–750 μm. The stroma is a collection of tightly packed collagen fibers that form bundles (fibrils) and plates parallel to the surface of the cornea. The main cellular representatives are keratocytes and immune cells. A statistically significant tendency of a decrease of the maximum density of stroma cells from the anterior sections (on average about 40,000 cells/mm^3) to the posterior sections towards the Descemet's membrane (on average about 20,000 cells/mm^3) has been established.

Keratocytes are mitotically resting motionless cells of mesenchymal origin, which have processes-keratopodias that provide contact with neighboring keratocytes, forming a continuously connected network (Fig. 1a, b). Keratocytes perform the function of producing and maintaining the extracellular matrix, ensuring strict morphostructural and biochemical constancy and transparency of corneal tissue. Keratocytes are unique cells of the neural crest that are involved in maintaining water balance and tissue homeostasis due to the synthesis of growth factors, building protein molecules, cytokines, neuropeptides and neurotrophins, metalloproteinase inhibitors [4].

The components of the extracellular matrix (ECM) of the corneal stroma include highly organized collagen (types I, III, V, VI, XII) and glycosaminoglycans (keratocan, keratan sulfate, decorin, mimecan and lumican), which are synthesized by keratocyte cells [6]. Keratocan and lumican are important glycosaminoglycans that are highly expressed in corneal keratocytes [7] and regulate its transparency and hydration balance, organizing and maintaining the topography of collagen fibrils [8].

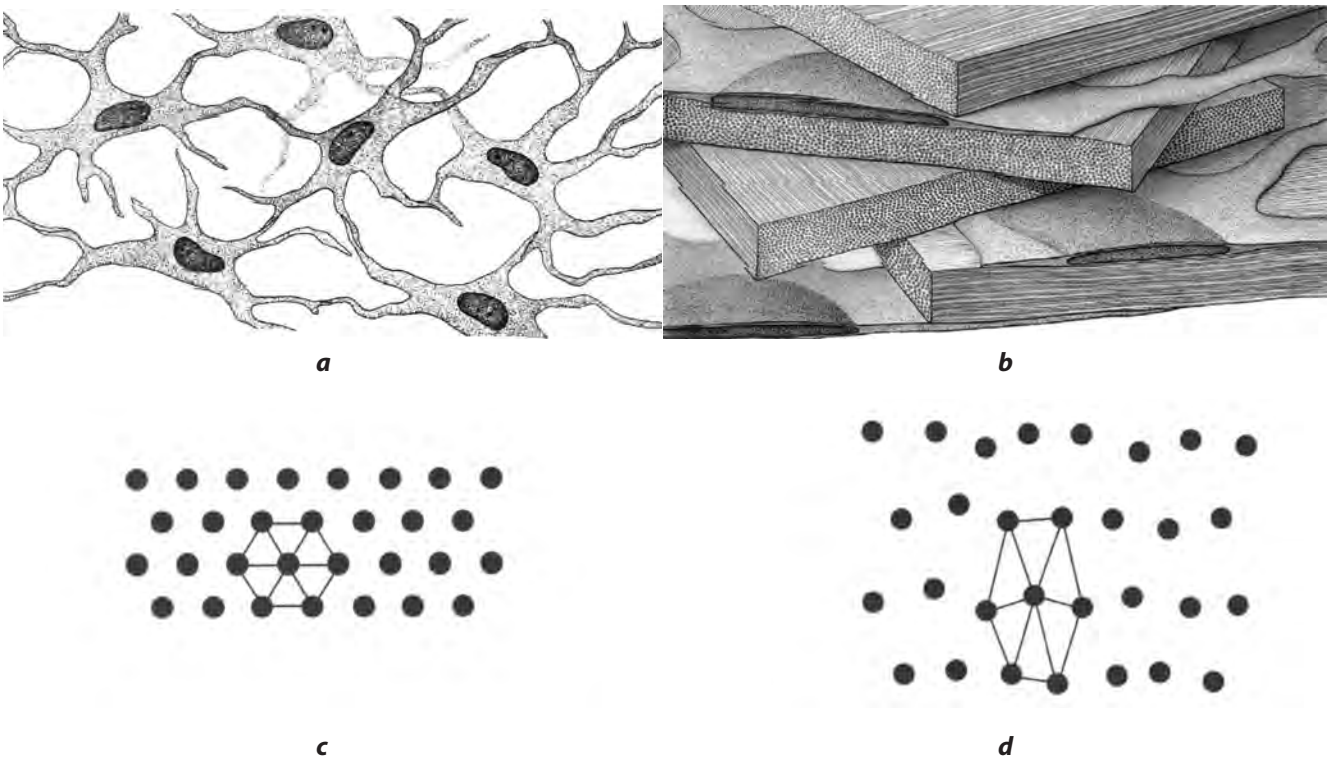


FIG. 1.

*General scheme of the corneal stroma structure: **a** – scheme of the cellular network of the corneal stroma; **b** – structure of dense fibrous connective tissue – collagen plates with thin flat cells between them which contact with neighboring cells by means of processes; **c** – scheme of orientation of collagen fibers – each fiber is located equidistant from the others; **d** – scheme of orientation of collagen fibers in an opaque cornea [5]]*

There are no blood vessels in the cornea, therefore nutrition and metabolism occur due to the vessels of the limbal network, moisture of the anterior chamber and tear fluid by osmosis and diffusion [9]. Metabolic processes are largely replenished by abundant innervation, which is represented by trophic, sensitive and vegetative nerve fibers, forming a perilimbal nerve plexus around the cornea. Entering the cornea, the nerves lose their myelin sheath and become invisible, which also ensures the transparency of the corneal tissue.

Injuries, infections and other pathological processes disrupt the organization of the stroma. Under altered conditions (with injuries, burns, surgical intervention, including the release of IL-1α, the tumor transforming growth factor β1.2 (TGF-β1.2)), part of the resting keratocytes located at the epicenter of the damage undergo apoptosis, forming an acellular zone in the area of damage [10]. Keratocytes bordering the site of injury extend new dendrite – like processes and migrate to the acellular zone before differentiating and proliferating into repair fibroblasts [11]. At the same time, keratocytes from the periphery also switch to an activated state, i.e. acquire the phenotype of motile contractile fibroblasts due to the reorganization of non-muscle myosin and smooth muscle actin α (α-SMA) into cytoskeletal stress fibers, migrating to the site of injury, proliferating and surrounding the wound in as a connected network of cells [12]. This physiological transformation of the phenotype is necessary to activate the mitotic cycle, increase functional activity and realize an active therapeutic effect – stromal wound healing. The observed increase in the size and number of organelles in cells and the acquisition of spindle-shaped cells reflects the enhanced synthetic activity of fibroblasts [13]. The fibroblast population can further differentiate into myofibroblasts characterized by α-SMA expression and an additional increase in stress fibers. Fibroblasts and myofibroblasts trigger regeneration processes in the cornea, causing rapid wound contraction due to the synthesis of a temporary matrix of opaque extracellular matrix [14].

The temporary matrix differs in composition and structure from the intact stroma. During regeneration, the extracellular matrix is synthesized by activated cells at an accelerated rate, in a less organized manner [15, 16]. A decrease in keratocan synthesis occurs in parallel with an increase in the production of decorin and chondroitin. This modified extracellular matrix promotes fibroblast migration; however, its altered biochemistry and structure can cause corneal opacity. Gradually, the temporary matrix is replaced by normal stroma components, including collagen types I and III, to restore the physiological function of the cornea. Thus, collagen expression increases in fibroblasts and reaches higher values in myofibroblasts compared to keratocytes [17]. In particular, high production of collagen I is characteristic of corneal fibroblasts and myofibroblasts, which is necessary for tissue remodeling [18].

Myofibroblasts are characterized by larger sizes compared to fibroblasts and express high levels of α-SMA, vimentin, and desmin [19, 20], which makes it possible to differentiate this cell population from others. α-SMA, vimentin and desmin are structural proteins involved in the formation of the cytoskeleton [21], a three-dimensional network inside the myofibroblast that determines the shape and mechanical support of the cell, provides movement and contractility [22]. The cell population of the corneal stroma has the ability to rapidly rebuild the cytoskeleton, which is directly related to its functional potential, since myofibroblasts acquire a high contractile ability necessary for physiological tissue remodeling. However, uncontrolled proliferation negatively affects the function of the stroma, disrupting the strictly ordered morphological structure of the tissue, thereby changing the transparency of the cornea. The question of the possibility of the reverse transition of corneal myofibroblasts to the original keratocytes under physiological conditions *in situ* is debatable and open. The possibility of reversion of myofibroblasts into fibroblasts has been shown when treating cells with low levels of fibroblast

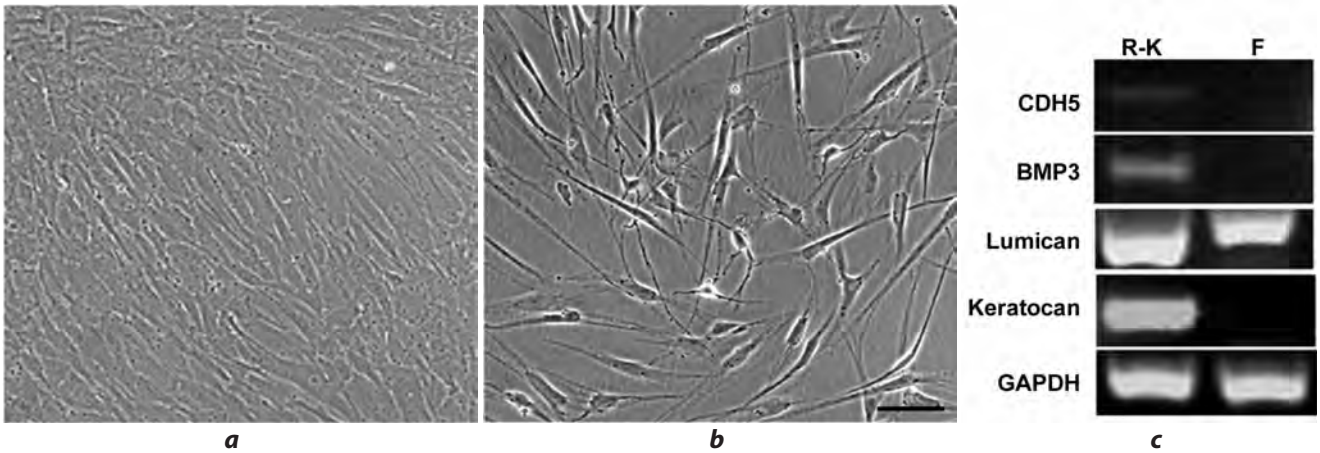


FIG. 2.

*Morphology and some markers of the expression of keratocytes and corneal stroma fibroblasts: **a** – morphology of fibroblasts (F); **b** – morphology of reversed keratocytes (R-K); **c** – expression of specific markers by reversed keratocytes and fibroblasts [14]]*

growth factor 1, 2 (FGF1, FGF2) [23]. This discovery supports the idea that corneal myofibroblasts and fibroblasts represent reversible phenotypes rather than terminally differentiated cell types.

One cell population that originated from another (Fig. 2a) under the influence of certain environmental conditions is called "reversed" population (Fig. 2b), that is, it has undergone a phenotypic transformation. Understanding the mechanisms that regulate the transformation of corneal keratocyte phenotypes is necessary for the development of tissue engineering of the corneal stroma.

Currently, numerous scientific teams are conducting research to determine the optimal conditions for the cultivation of corneal cells. There are several ways to isolate corneal stromal cells, which include mechanical (removal of epithelial and endothelial layers) and prolonged enzymatic treatment of the cornea [24, 25]. Usually, cadaver and unsuitable for transplantation human corneas are used to isolating cells. The disadvantages of the known methods are the complexity and insufficient «purity» of the cell population, since the result is a mixture of cells containing a non-homogenous population from the anterior, middle and posterior parts of the stroma, as well as an admixture of epithelial and endothelial cells of the corresponding layers of the cornea. Populations of cells derived from the stroma of bovine, pigs, rabbits and mice are used as a cell source alternative to the human eye stroma. In culture with the addition of serum, corneal stromal cells acquire the phenotype of fibroblasts, in a serum-free environment with the addition of insulin, transferrin and selenium (ITS), on the contrary, retain the phenotype of keratocytes [26], and in the presence of ascorbic acid – produce and accumulate collagen and proteoglycans, which mimics their functions in the native cornea [27].

A detailed study of the characteristics of corneal stroma cell populations is carried out in order to verify, determine the intensity and nature of the potential therapeutic effect. One of these characteristics is the determination of the phenotypic properties of a cell population, that is, the determination of specific expression markers that distinguish one cell population from another (Fig. 2b).

SECTION 2. PATHOPHYSIOLOGICAL MECHANISMS OF CORNEAL TRANSPARENCY DISORDERS

Keratocytes account for about 3–5 % of the total stromal volume [28]. Figure 3 shows the histological structure of the cornea, in whose own substance a low cell content is clearly determined. This ratio is necessary to maintain the transparency of the cornea and is explained by the peculiarities of light transmission. The cytoplasm of a mitotically resting keratocyte scatters the light beam to a less-

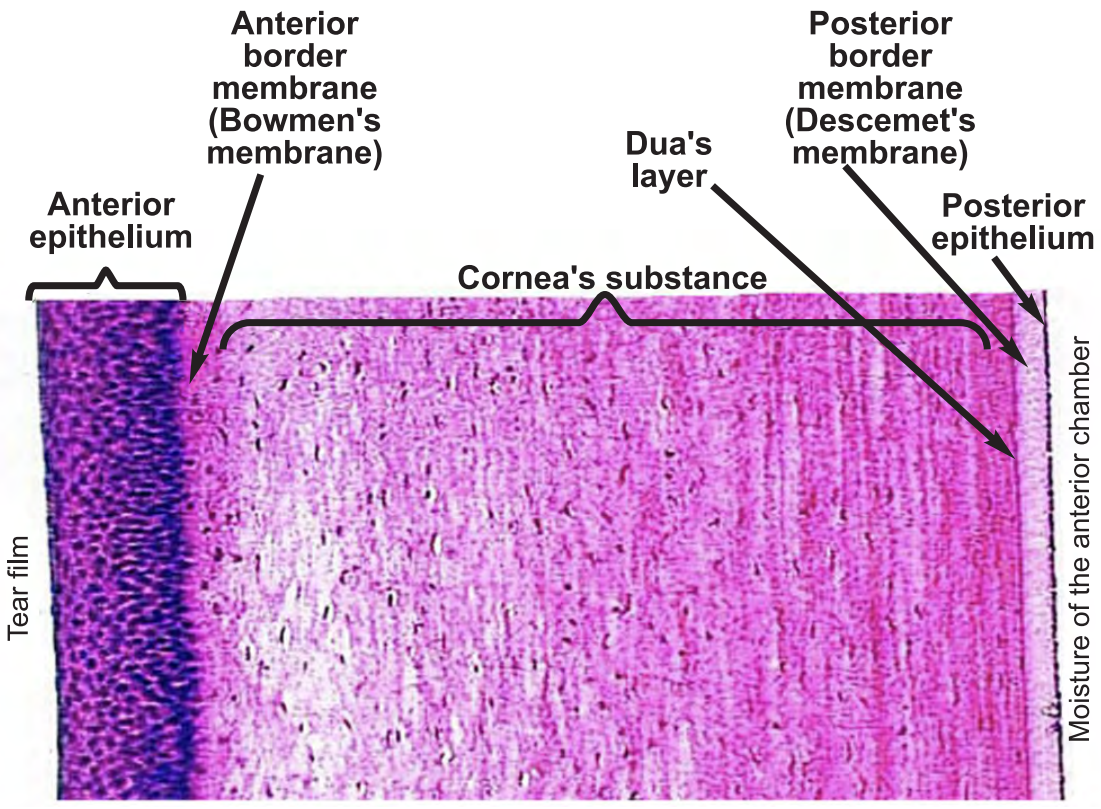


FIG. 3.
Histological structure of the cornea (hematoxylin and eosin staining) [30]

er extent due to the low content of organelles in cells [29], due to which the unique structure of the cornea determines its functional role.

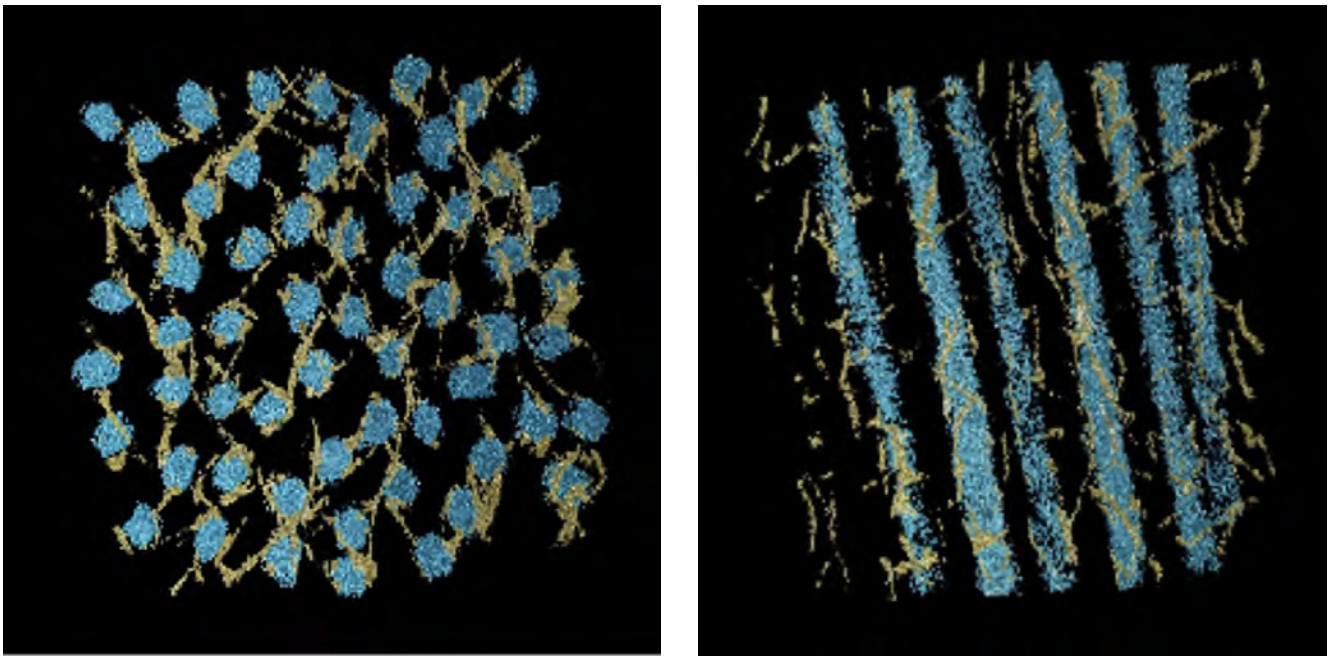
Functional activity at cell (keratocyte) level may vary for a number of reasons, which may be exogenous, iatrogenic (trauma, infections, etc.) or endogenous. Endogenous causes include: cell autoregulation disorders (for example, hereditary or acquired enzyme defects); transport systems disorders leading to tissue (cell) hypoxia; disorders of endocrine or nervous regulation of trophism [30]. By influencing the cellular microenvironment, using a wide range of biologically active substances, it is possible to influence the listed endogenous causes of impaired functional activity of the stroma population. One of the ways of such influence may be a transplantable cell population of stromal cells. Thus, the ability of donor stromal cells to repopulate recipient tissue without scarring has been proven (follow-up time is 30 years) [31].

Impaired functional activity of keratocytes has been found to result in changes in corneal transparency [32]. This fact is explained by the role of keratocytes in the synthesis of extracellular matrix components – collagen and glycosaminoglycans; regulation of the constancy of the water and electrolyte balance of the tissue directly – realizing the cellular membrane transport of substances, electrolytes and water into and out of the tissue, and indirectly – by synthesizing hydrophilic components of the extracellular matrix, which «attract» water molecules [33]. These processes are responsible for creating a strict spatially ordered structure of the corneal tissue through which light passes (the interference process). The physical reason for the disruption of the corneal transparency is an increase in the light scattering [34].

There are five main causes of increased light scattering in the opaque cornea:

1. Changes in the structure of collagen fibers, disruption of their spatial hexagonal arrangement and/or thickening of fibrils due to fusion

In a healthy transparent cornea, collagen fibers assemble into bundles called fibrils (lamellae), which are of the same size and strictly oriented in space relative to each other to minimize the scattering of the light beam. This alignment of collagen fibers in the cornea is ensured by glycosaminoglycans that line up around the fibrils (Fig. 4a, Fig. 5a) and create «bridges» between them (Fig. 4b, Fig. 5b) – equal inter-fibrillar distances [35]. Since glycosaminoglycans are hydrophilic compounds, they attract water molecules to themselves and thereby perform their function. If the position of the collagen fiber is disrupted, osmotic and electrostatic pressure forces are applied to it and return the fiber to its place (Fig. 5b, d) [36]. The strict morphological structure is also ensured by the uniform diameter of the fibrils and their ordered organization, which creates conditions for the passage of a light beam through the cornea to the retina of the eye, i.e. realizes the transparency of the corneal tissue.



a ***b***

FIG. 4.

Spatial orientation of glycosaminoglycans and lamellae: ***a*** *– glycosaminoglycans around lamellae (indicated by short thin lines);* ***b*** *– glycosaminoglycans in the shape of "bridges" between the lamellae [35]*

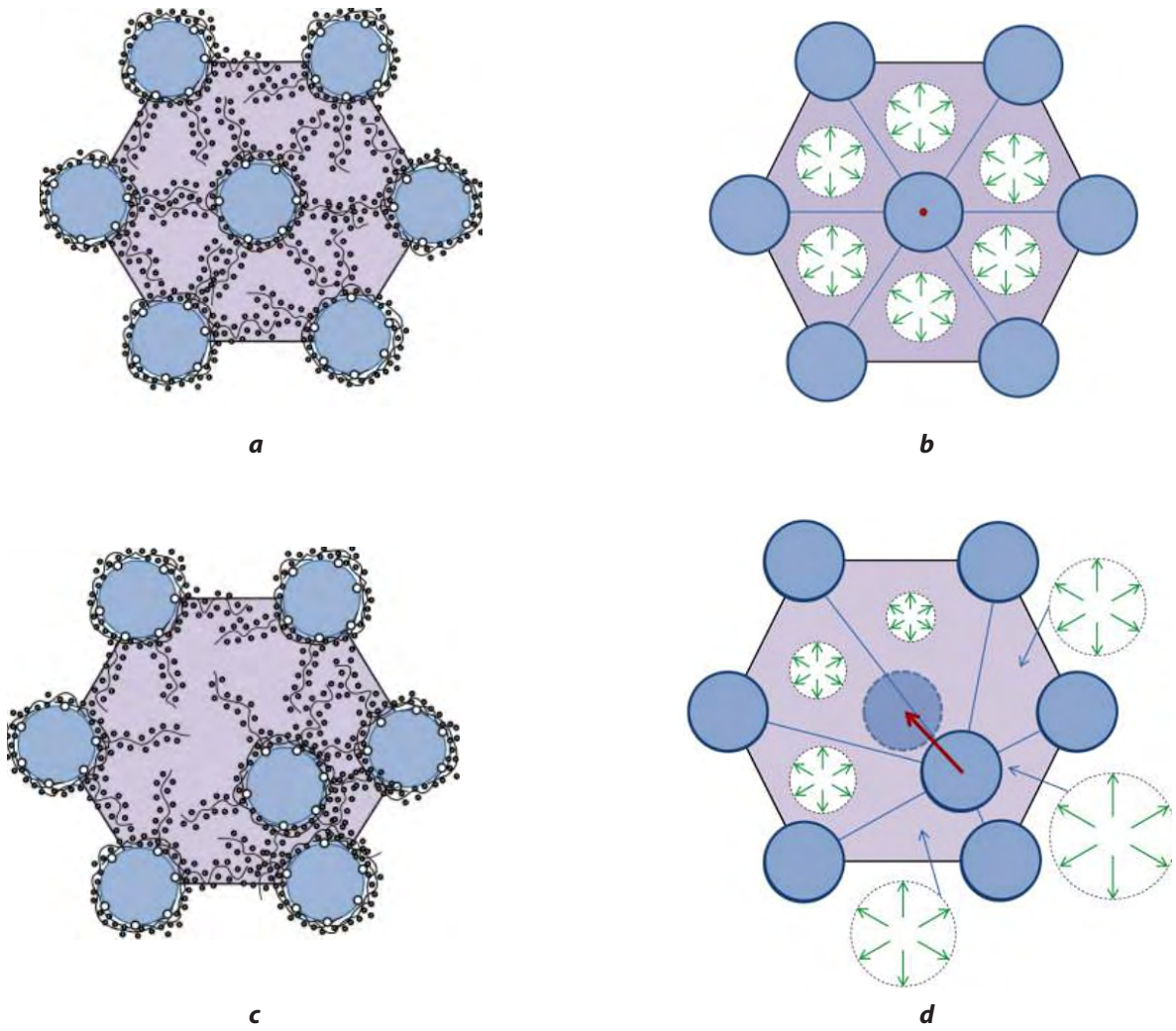


FIG. 5.

*The action of osmotic (**a**) and electrostatic (**b**) pressure at the displacement of the collagen fiber (**c**, **d**) [36]*

2. Transformation of keratocytes into fibroblasts and myofibroblasts ("activated" cells)

Keratocyte nuclei have high light scattering indicators. Therefore, the cornea would not be transparent if there were a large number of cells in the stroma, whose nuclei would not allow the light beam to pass through this connective tissue structure of the eye. The transition of resting keratocytes to a reparative phenotype is associated with profound molecular, biochemical and morphological changes. Activated stroma cells have a high degree of light scattering, thereby directly capable of causing a disruption of corneal transparency. Thus, a confirmatory fact of higher light scattering in the population of myofibroblasts compared with the population of corneal keratocytes was established [37].

3. Synthesis of fibrous opaque extracellular protein matrix by "activated" corneal cells

In response to trauma, surgical intervention (after excimer laser ablation for photorefractive keratectomy (PRK)), the influence of infectious agents (viruses/bacteria), as well as in genetically determined abnormalities and congenital corneal dystrophies, resting keratocytes become mitotically active and differentiate into fibroblasts and myofibroblasts, excessively synthesizing new proteins such as fibronectin, α-SMA, tenascin-C, collagen types III, IV, fibrillin 1 and others that disrupt the organization of fibrils, which leads to corneal opacity [38]. Thus, the main reason for the disruption of corneal transparency during activation of the stroma cell population is a change in the spatial arrangement of collagen fibrils due to the synthesis of excess protein matrix. The fibrous opaque extracellular matrix is reversible with the transition to the normal architecture of the corneal stroma during the "rejuvenation" of the keratocyte cell population, which is an important strategy for restoring tissue transparency [39].

4. Edema of the corneal stroma

Stroma edema leads to the corneal tissue opacity and can be caused by various factors: in case of disruption of the barrier structure integrity – Bowman's or Descemet's membranes – after injuries, infectious effects, acute keratoconus (hydrops), surgical interventions

and infectious lesions, corneal dystrophy. The mechanisms of "hydration" of the cornea can also include a disruption of cellular transmembrane or passive water transport in the corneal stroma and a disruption of the synthesis of protein hydrophilic components of the intercellular matrix with changes in the functional activity of the keratocyte, pathologies of the membrane-pumping function of the endothelial (for example, Fuchs syndrome) and epithelial barriers (recurrent erosion/corneal ulcer).

5. Decrease in the level of crystallin expression by keratocytes of the stroma

Crystallins are water-soluble proteins with enzymatic activity expressed by keratocytes of the stroma, epithelial cells of the cornea and lens [40], their role is in antioxidant protection and reduction of light scattering of transparent structures of the eye [41, 42]. The antioxidant role of corneal crystallins is protection from damage by peroxide compounds and free radicals, including those formed from exposure to UV radiation. The family of aldehyde dehydrogenases (ALDH3A1, -1A1, -2, -7A1, -1B1) and transketolase (TKT) belong to the corneal crystallins [43]. With a decrease in the concentration of crystallins in the cornea (TKT and ALDH1A1), there is an increase in light scattering, i.e. a decrease in its transparency is noted. The conversion of keratocytes into a reparative phenotype (fibroblasts and myofibroblasts) in the corneas of rabbits and bulls leads to loss of crystallins, which is also confirmed in cultural models [44].

Thus, corneal opacity can be caused by one of the listed pathogenetic changes, or it can be a consequence of the combined effects of several factors. The main processes of regulation of tissue homeostasis consist primarily in maintaining a strict morphological structure of the cornea.

SECTION 3. THE ROLE OF CYTOKINES AND GROWTH FACTORS IN CORNEAL STROMA REPAIR

Stromal stem cells realize the effect of tissue repair due to active interaction with the microenvironment due to the secretion of cytokines, growth factors, neuropeptides and neurotrophins [45].

The loss of functional activity of stromal keratocytes is the loss of the ability of the cornea to self–renewal. In the pathogenesis of many pathological changes of the cornea, such as post-burn, post-traumatic, ectatic conditions (including keratoconus), a special role is assigned to the loss of keratocytes caused by an increase in apoptosis [46, 47]. The inflammatory theory is one of the modern explanations of this process; modern scientists are trying to explain the decrease in the elastic properties of corneal tissue in keratoconus by the same fundamental processes at the cell level, i.e. the effect of free radicals and oxidative stress products on the tissue. It was found that stroma cells from patients with keratoconus form metabolites that indicate oxidative stress of the cell in both 2D and 3D cultures [48]. Deceleration in the progression of ectasia in a patient with keratoconus with the use of cyclosporine A, an immunomodulatory agent, was also reported [49].

The reparative potential of stromal keratocytes is feasible due to its ability to reverse transition into a less differentiated class of cells (the process of reverse cytodifferentiation). According to modern concepts, one of the reasons for the transition of keratocytes to an activated state is the effect of TGF-β on them [50]. The release of TGF-β occurs under adverse conditions, with damage to the epithelium and Bowman's membrane as a result of injury, surgery, exposure to chemical or infectious agents, or other pathological conditions.

In various organs, including the cornea, members of the TGF-β family are key regulators of fibrosis and scarring through signaling mechanisms (TGF-β/Smad-signaling) via signaling mRNA, as well as through other signaling pathways [51, 52]. The TGF-β family consists of three closely related isoforms (β1, β2 and β3) that play different roles in cell differentiation and tissue regeneration. Thus, TGF-β1 and -β2 mediate tissue fibrosis and scar formation [53, 54], unlike TGF-β3, which acts as an inhibitor of scarring processes [55]. The same mechanisms of interaction have been determined for the cornea. Thus, TGF-β1 and TGF-β2 contribute to scarring of the stroma [56], while TGF-β3 has been proven to restore corneal transparency [57].

The complex of the signaling molecule and receptor SDF1/CXCR4 (stromal cell derived factor-1) is expressed on corneal fibroblasts, participating in regeneration processes. The role of the complex is to organize the extracellular matrix, accelerate the migration of mesenchymal stem cells (MSCs) [58], increase the expression of α-SMA in fibroblasts, promoting the transition of fibroblasts to myofibroblasts, increasing tissue scarring [59]. It has been suggested that the activation of stem cell homing and secretion of growth factors through the chemokine axis SDF-1/CXCR4 [60]. The expression of the complex increases in response to an increase in the concentration of hypoxic factor HIF-1a and mechanical damage, which leads to increased migration of stem cells through chemotaxis. The antihypoxic role of the complex, the release of which occurs during radiation exposure to the tumor (immunological effect), has been established [61]. An increase in the expression of the SDF1/CXCR4 complex stimulates the effective migration of stem cells to the injury zone, recruitment of fibroblasts, and activation of endogenous repair processes [62].

Intermediate filaments are important elements of the cytoskeleton for the regulation of processes related to tissue repair. Fibronectin is a protein highly expressed on corneal fibroblasts, which is involved in the organization of the extracellular matrix during regeneration. It forms pathways-channels to accelerate migration and create a dense cellular network – the spread of cells to the site of injury [63]. Vimentin, like desmin, are intermediate protein insoluble filaments of the cy-

toskeleton of stromal keratocytes, fibroblasts and myofibroblasts [64, 65], whose role is to carry out the transition of the stroma cell population to myofibroblasts [66], as well as to accelerate the processes of proliferation and migration of fibroblasts to the site of the «wound» [67], thereby providing stroma remodeling after injuries, surgical interventions, etc. An increase in vimentin expression in stromal cells after surgery (PRK) and a decrease in the rate of fibroblast migration to the wound site in the stroma in mice with vimentin deficiency were established [68]. Fibroblasts and myofibroblasts have also been found to maintain higher levels of vimentin than keratocytes [69].

Insulin – like growth factor 1 (IGF-1) is a protein of the family of insulin-like growth factors, responsible for maintaining corneal homeostasis; regulates the formation of a communication network between keratocytes [70], proliferation and differentiation of keratocytes into fibroblasts and myofibroblasts during inflammatory processes and damage [71]. T. Sarenac et al. showed that IGF-1 increases the secretion of keratocan, lumican and cytosolic crystallin (ALDH3A1). IGF-1 reduces the likelihood of scar formation in the corneal stroma, increasing keratocyte proliferation and affecting wound healing [72].

Proinflammatory cytokine – tumor necrosis factor α (TNF-α) – and soluble intercellular adhesion molecule-1 (ICAM-1) play an important role in the regulation of inflammatory reactions in infectious and non-infectious processes (in allergic reactions) in the cornea, are expressed on corneal keratocytes and fibroblasts [73], ensure the migration of macrophages and leukocytes, regulate the processes of infiltration and activation of polymorphonuclear neutrophils in the focus of inflammation [74].

Erythropoietin (EPO) is a glycoprotein that is an active humoral factor that regulates the growth and development of various cells, tissues and organ systems. EPO not only stimulates the proliferation and differentiation of erythroid precursor cells, but also has antiapoptotic and antioxidant effects, participates in neuroprotection and angiogenesis, and increases cell survival in hypoxia. EPO expression has been shown on many cells, including corneal keratocytes of mice. The connection of this cytokine with the processes of neovasculogenesis of the eye has been established [75]. High levels of EPO have been found in vitreous samples from patients with proliferative diabetic retinopathy, however, the role of EPO in healthy cornea is largely unknown.

Neurotrophic factors (NGF, NT-3, BDNF) and tyrosine kinase receptors (TrkA, TrkB, TrkC and TrkE) are a number of compounds that are synthesized in the epithelium and stroma of the cornea, are able to influence each other, activating the processes of migration and proliferation and the ability to regulate the function of cytokine exchange inside the cornea [76].

Corneal sensitivity is provided by the ocular branch of the trigeminal nerve, which causes protective reflexes such as blinking and lacrimation. The corneal nerves depart from the ocular branch of the trigeminal nerve and provide mechanical, chemical, thermal sensitivity, as well as perform a trophic function due to the release of nutrients and trophic factors. Local and systemic conditions caused by trigeminal nerve damage (such as diabetes mellitus, dry eye syndrome, keratitis with herpes simplex virus, neurotrophic keratitis, etc.) are associated with impaired corneal innervation, decreased tear production and impaired healing of epithelial and stromal wounds. Corneal nerves express several neurotransmitters, including substance P (SP) – calcitonin gene-related peptide (CGRP), acetylcholine, cholecystokinin, norepinephrine, serotonin, neuropeptide Y (NPY), vasointestinal peptide (VIP), methencephalin, natriuretic brain peptide, vasopressin and neurotensin. It was demonstrated that SP is able to modulate the proliferation and migration of corneal cells and their adhesion. The use of SP associated with IGF-1, as well as nerve growth factor (NGF), epidermis growth factor (EGF), vascular endothelium growth factor (VEGF), semaphorins, neurotrophins 3 and 4 (NT-3, NT-4), which increase the rate of corneal healing and stimulate the adhesion of epithelial cells was demonstrated [77, 78]. Conversely, corneal stromal and epithelial cells secrete neuropeptides, neurotrophins and growth factors that affect survival, differentiation of nerve fibers and their maturation, including NGF, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophins 3, 4, 5, EGF and glial cell-derived neurotrophic factor (GDNF) [79, 80].

Thus, corneal cells and the ocular branch of the trigeminal nerve are the main participants of the interaction during corneal tissue repair at the neurotrophic level. They are able to mutually activate each other to produce cytokines, neuropeptides, neurotransmitters and growth factors to improve trophic function and accelerate corneal wound healing. Consequently, all local and systemic conditions leading to damage to the corneal sensory nerve can affect this interaction, causing a disruption of corneal repair and healing rate. New compounds capable of stimulating corneal nerve repair are under development. Among them, eye drops with nerve growth factors (including platelet-rich plasma) proved to be safe and effective for stimulating healing and improving corneal sensitivity in patients with neurotrophic keratitis.

CONCLUSION

New knowledge about the role and interaction of the extracellular matrix and the corneal stroma cell population in maintaining tissue homeostasis is important for understanding the tactics of treating many diseases and purposefully influencing the processes of repair of the connective tissue structure. In recent years, ideas about the extracellular matrix, which was previously considered only from one side – as architectonics, support for cells and tissues, have changed significantly. Numerous studies confirm that the extracellular matrix is a physiologically active participant in living tissue, which is responsible for the most important

processes of cell and tissue life. The cellular population of the corneal stroma also plays a crucial role in ensuring the physiological repair processes of the tissue. Mutually influencing each other, the connective tissue components of the stroma create a strict morphological structure that ensures the main property of the cornea of the eye – transparency.

A promising alternative way to eliminate corneal blindness is stem cell therapy, which is etiotropic in nature, due to the activation of various signaling pathways to tissue regeneration, solving two primary tasks: replenishing the lost population of keratocytes and restoring its functional role (production of extracellular matrix, synthesis of cytokines, growth factors, neuropeptides, etc.). An understanding of the modern anatomical and physiological foundations of the structure of the cornea, described in this literature review, will help to approach the study of this topic in order to determine the «application points» of potential therapeutic agents.

Due to the feedback between cellular elements and their microenvironment, which evolves during tissue development, a unique molecular composition of the extracellular matrix is formed, which has a powerful effect on biochemical and biophysical processes in cells and determines cell-matrix (epithelio-stromal) interactions. The term «corneal homeostasis» combines a whole complex of intercellular and intermolecular interactions, the study of which from the point of view of the main processes of neuro-humoral regulation is necessary for qualitative external influence on the processes of corneal tissue restoration.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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