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THE SIXTH LAYER OF THE CORNEA: IS IT FICTION OR REAL FACT?

Summary. *There are many facts pointing to the special status of the corneal stroma adjacent to Descemet's membrane. Morphological study of the process of regeneration and experimental embryology data show that the posterior section of the corneal stroma laying in the back of Descemet's membrane is a special layer having embryogenetic and histogenetic nuances and relationships that distinguish it from the rest corneal stromal tissue.*
Key words: *corneal stroma, Descemet's membrane.*

The aim of our work is to show real place of the posterior part of corneal stroma in eye pathology and physiology. This will come close to developing the concept of a single collagen-elastic eye capsule comprising the membrane Dua, lamina cribrosa and the Bruch membrane. Violation of this membrane homeostasis may lead to various diseases: keratoconus, glaucoma, macular degeneration.

Centennial path of corneal transplant surgery is characterized by improving the methods of operations relating not only to the eye of the patient, but also the actual cornea donor. Of course, corneal transplantation of an entire thickness - penetrating keratoplasty - remains the gold standard in corneal transplant surgery. However, such radical surgery is fraught with several disadvantages, namely the long duration of recovery, increased risk of rupture in the area of surgery, development of astigmatism and complications of transplant engraftment. At present, we have the opportunity to perform corneal layer replacement of posterior segment of cornea in some pathological changes. Thus, the replacing of posterior epithelium (endothelium), which called DSEK (Descemet Stripping Endothelial Keratoplasty), is supplanted by penetrating keratoplasty in many cases.

Due to the fact that the cornea is a part of the outer layer of the eye, it is exposed to adverse environmental factors. Cornea is easily involved in the pathological process and slowly comes out of it as it has no blood vessels, and all metabolic processes are slow. According to the statistics, of all the patients who come to outpatient care every fourth has a disease of the cornea. The social significance of corneal pathology is due not only to high-speed development of the disease, but also the duration of treatment, frequent relapses and reduced visual acuity. Also, corneal diseases are a major cause of blindness and occupy 25-30% of all eye diseases.

Numerous innovations in eye surgery technology and tools, especially in the last decade, made it possible to improve the methods of corneal transplantation which helped to solve some of the problems listed above. For any damage to the corneal epithelium its replacement can be performed during the procedure called DSEK - Descemet Stripping Endothelial Keratoplasty or DMEK - Descemet Membrane Endothelium Keratoplasty. Endothelial transplantation allows to restore vision quickly, it don't weaken eyesight, astigmatism is a neutral, and the frequency of graft rejection has reduced. Today Deep Anterior Lamelar Keratoplasty - DALK is the optimal lamellar keratoplasty, it's performed by method "air bubbles" or BB - big bubble. During this operation an attention was drawn to the small (up to 15μm), but rather dense structure placed between the posterior membrane and the rest of cornea stroma.

As a result of observations of DALK, three years ago, a professor of ophthalmology and optics of the University of Nottingham together with colleagues announced the discovery of a new - sixth layer of the cornea called Dua`s layer. For the opening of this layer the technique of BB, offered by Mohamed Anwar, contributed. H. Dua and his colleagues found this layer in the process of DALK with "BB" in the experiment on enucleated human eyes. Histological study conducted by Dua and his colleagues using light and electron microscopy showed that the back wall of BB is formed by a thin layer of collagen fibers (Dua`s layer), the posterior (Descemet) membrane and endothelium of the anterior chamber of the eye (Descemet's endothelium). Collagen fibers of adjacent stroma of the cornea penetrate the layer of Dua. The average thickness of Dua`s layer - 10.15±3.6 μm (range 6.3 to 15.83) that is comparable in average thickness to the posterior membrane - 10,97±2,36 μm (range 7.8 to 13 98). Dua's layer is formed of 5-8 bundles of collagen fibers, tightly packed in a thin plate. The posterior membrane of cornea has the same structure in the area of BB as well as in control zones of unfilled air. Collagen fibers are oriented in the longitudinal and transverse directions and parts of them in an oblique direction. The posterior membrane of cornea showed long collagen fibers that connects to Dua`s layer [1].

Sometimes, damaged bundles of collagen fibers was found on the front surface of Dua's layer as accumulations of the collagen's substance peeled off from its own cornea. For comparison, according to the width of the stroma of the cornea in front of the Dua's layer there was showed only 3-5 bundles of collagen fibers in control areas of unfilled air. The diameter of the collagen fibers in the Dua's layer ranges 21,70±2,43 nm, and corneal stroma adjacent to the layer Dua within 24,20±2,68 nm. Dua and his colleagues showed that this difference is statistically significant. They also found that the distance between the fibers in the Dua's layer compouds 9,64±7,74 nm, and the corneal stroma 10,09±7,91 nm. However, if the distance between the fibers is measured

in the centers of adjacent fibers, the space between the fibers thinner in Dua's layer than in the stroma of the cornea. According Dua and his collaborators, it causes of greater filling of proteoglycans and may contribute a gel-like structure, that may explain why it is impervious to air that promotes the formation of BB.

By the method of scanning electron microscopy there is found that the front surface of Dua's layer is arranged by parallel bundles of collagen fibers, while the posterior surface is formed by coarse bundles of collagen fibers and differs from the main stroma of the cornea and the posterior membrane of cornea. Bundles of collagen fibers connect space between the Dua's layer and the stroma of the cornea as it is evidenced by the data of scanning and transmission electron microscopy. Unlike stroma of cornea, Dua's layer, which forms the back stratum of BB, does not contain keratocytes.

Microscopic studies of the cross cut of the edge of the cornea in place of its transition to a limb establishes that Dua's layer, which is a thin plate of collagen fibers in front of the posterior stratum of cornea, passes on the periphery and outside of Descemet's membrane, firstly in the form of the thick strips, which then expands in form of triangle, and further the distance between collagen fibers become increased with approach to the sclera, ciliary body and root of the iris. There were revealed with scanning electron microscopy researches that the bundles of collagen fibers passed radially out from the front surface of the peripheral edge of the dentate line named Schwalbe's zone, and wrapped around the periphery of posterior membrane. However, when the posterior membrane is peeled off from its own corneal substance the Dua's layer is visualized as a smooth sheet, which at the periphery of the cornea is oriented radially and stratified into broad bundles of collagen fibers, which in turn are divided into thin bundles of collagen fibers. These fibers are connected and intertwined with similar arranged adjacent beams moving in the direction of the iris and ciliary body, and create trabecular meshwork (TM). These observations are confirmed by using of transmission electron microscopy (TEM) [2].

TEM of the peripheral part of the cornea confirms that Dua's layer goes outside the posterior membrane. Compact lamellated location of Dua's layer begins to open about 350 microns closer to the center of the posterior membrane. Collagen fibers defined in the periphery of the cornea in Dua's layer are an average of 322 microns (ranging from 260 to 390 μm) near the center of the end of the posterior membrane. Here they are related with basement membrane fibers that separate Dua's layer. Attaching of the collagen fibers to the basement membrane and between them there are in the periphery of Dua's layer. The morphology of cells arranged in Dua's layer is different from keratocytes which located ahead of the posterior membrane and in the stroma of the cornea. The core and trabecular cells of keratocytes are stained by using DAPI staining (nuclear stain). However trabecular cells are negative for CD34 (keratocytes marker) which stains all keratocytes in the stroma of the cornea. The presence of cells in the trabecular layer corresponds Dua's opinion according to which most of the trabecular beams that form the trabecular meshwork behind the scleral venous sinus are formed from collagen fibers that go from Dua's layer. Front collagen fibers, along with some collagen fibers of the corneal limbus, form anterior wall of the scleral venous sinus. Bundles of collagen fibers described in Dua's layer are widely distributed in the trabecular meshwork. Elastic fibers are arranged in a grid of the trabecular meshwork, but they are not found within Dua's layer. It should be note that on the free surface endothelium has villi and cilia, which brings him to the trabecular cells and said their community [3].

According Dua and his co-authors opinion the division of bundles of collagen fibers of Dua's layer on the broad and narrow beams in the periphery of the cornea and their relationship with bundles of collagen fibers of trabecular meshwork, which is determined during the scanning electron microscopy and transmission electron microscopy establishes that the trabecular meshwork is continuation of Dua's layer. Scanning electron microscopy of Dua's layer after detachment of the posterior membrane shows that collagen fibers arranged in Dua's layer are continuing broad beams of apical trabecular meshwork. The cells within the peripheral part of the cornea in Dua's layer are surrounded by a basal membrane with which they form contacts within Dua's layer. The rays of the trabecular meshwork is known to be covered the basement membrane which is attached to the endothelial cells. The presence of these cells together with basal membrane in the periphery of Dua's layer suggests that the forming of the trabecular meshwork beams starts in Dua's layer about 500 microns closer to the center of the ending of Descemet's membrane. Staining for laminin, which is part of the basement membrane, is strongly positive in the trabecular meshwork and in the periphery of Dua's layer that confirms the above observation. Ray bundles of the collagen fibers of trabecular meshwork are deprived the cells. This is consistent with the conclusion that there are no keratocytes in Dua's layer. This indicates that the formation of the trabecular meshwork begins in the peripheral part of the cornea, before the ending of Descemet's membrane and not aside of Descemet's membrane. As Dua's layer is thinner toward the periphery, the distance between the last keratocytes and posterior membrane is reduced compared to the central part of Dua's layer.

Thus, while the bulk of the corneal stroma is merged at the periphery with other scleral stroma, bundles of collagen fibers of Dua's layer continue in trabecular meshwork. The significance of these observations and the importance of the structure of the trabecular meshwork in the development of glaucoma and blindness, according to opinion of Dua and his co-authors, should define researches of the attitudes between the structure of cornea and trabecular meshwork. It is found that in patients with keratoconus Dua's layer is stretched with posterior membrane, and acute hidropsiya

develops not only through the gap of Descemet's membrane, but through gap of Dua's layer. Rupture of the posterior membrane and Dua's layer, leading to keratoconus, there is obvious cause of sudden stromal hydration than just the break of the posterior membrane. Also it is found that descemetocele, which are believed to be the resistance to perforation, supported by strength of Dua's layer. When the Dua's layer damaged or destroyed by proteolytic enzymes, Descemet's membrane quickly get perforation. Some facts demonstrate the presence Dua's layer that covers the posterior membrane in descemetocele.

Macular dystrophy that manifests as clouding of the whole corneal stroma also has an impact on the structure of Dua's layer. In the course of performance of DALK by BB in macular degeneration of cornea, it is found residual opacity in the saved Dua's layer. Some surgeons as usual leave them and complete the operation, but the others carefully cut through Dua's layer (BB converts from Type 1 into Type 2), to remove all areas of turbidity. Thus, when macular degeneration, BB type 2 may be more appropriate, although at present the advantage of removing of Dua's layer in these patients isn't demonstrated. By our data and wide experience of DALK surgery (Dr. Tarek Katamish, Cairo), when we remove diseased corneal stroma by using the techniques of big bubbles, in the majority of patients with postinfectious scarring of the cornea it is found a clear Dua's layer. This interesting observation can be explained by the lack of keratocytes in this layer, which in the case of infection and inflammation differentiate in myofibroblasts and fibroblasts and form a scar [6].

Thus, according to opinion of H.Dua and his collaborators, information on Dua's layer is important for understanding the pathology of the cornea. The understanding of the biomechanical properties of Dua's layer in terms of its strength, flexibility and elasticity encourages surgeons to perform the innovative surgical procedures. So, after removal of the corneal stroma by the method of "big bubble" in cases of corneal scarring surgeon Dr. Ahmad Atef from Cairo has successfully completed the procedure of phacoemulsification with implant lens in two patients. This procedure is called DALK-triple procedure, and clinical application is direct understanding of the biomechanical properties of strength of Dua's layer. It remains to be determined as this layer affects the biomechanical properties of the cornea. Unlike Dua's layer, trabecular meshwork also has a network of elastic fibers that are connected with the tendon attached to the ciliary muscle and bundles of collagen fibers in the corneal stroma . It is believed that the tone of the ciliary muscle may directly affect the bundles of fibers of trabecular network and regulate aqueous humor. Biomechanical properties of the cornea and sclera is known to be influence the pathogenesis of glaucoma. The cornea is the subject of constant stress associated with movements caused by blinking, rubbing and pulsation of eyes. It is likely that these factors influence the structure of Dua's layer and trabecular meshwork. Recent researches have shown that risk of increase of intraocular pressure and the secondary glaucoma is higher at patients who have penetrating keratoplasty in comparison with DALK.

According to opinion of Dua and his co-authors, mentioned clinical monitoring caused enough doubt in the concept of "Descemet stripping" in DALK procedure. These led to the assumption that there is a separate layer in the stroma of the cornea adjoining to Descemet's membrane which is located between the front surface of the posterior membrane and deep own corneal stroma. This layer provide plane of cleavage between it and the deep corneal stroma in procedure of DALK. This hypothesis was proposed and initial clinical and histological evidence of existence of Dua's layer were presented at the Annual Congress of the Royal College of Ophthalmologists UK in May, 2007. Further evidence has been presented at the Societa Italiana Cellule Staminali e Superficie Oculare (VI Congress S.I.C.S.S.O. Lecce, 14-16 June, 2007).

Despite a recognition of opinion of Dua and colleagues, a part of scientists calls into question presence of new layer in cornea. So, Peter McDonnell the employee of Johns Hopkins University and Medical Editor of Ophthalmology Times noted that time is necessary to make sure that other researchers confirm the existence of this "new layer" and to determine its potential significance. He added that his personal vision of the news about the opening of a new layer is that results are not describing a new layer in the sense that investing in the concept of "layers of the cornea".

Roger Staynert from the University of California described the new layer as "a false discovery" and criticized the choice of the name for this layer, noting that current prevailing trend when scientists avoid naming anatomical structure and physiological processes by name of author, instead try to reflect the title of the essence of a subject or phenomenon. He also emphasized that described Dua phenomena were known before and not seen as signs of an additional layer, only just as individual structural features of the stroma of the cornea.

In February, 2014 MacKay and colleagues said they met this recent news about the opening of a new layer of the cornea with skepticism and distrust. As Roger Steiner they stressed that the existence of pre-Descemet stromal tissue that remains after pneumo dissection is well known. Their further investigation of this stroma confirmed that it is only part of stroma, but not a new layer. And also he has expressed critical opinion that the investigator allegedly called the opened layer by own name and emphasized that it is contrary to the present trend in medicine to avoid eponyms. Thus we can conclude that Dua and his colleagues have drawn attention of ophthalmologists, surgeons and scientists to extremely important issues that undoubtedly will lead to progress in the treatment of pathological processes in the cornea. However, according to some authors, it is not enough the presented proofs for a recognition of a new layer of a cornea.

Vladimir Filatov and August Wagenmann were the first who described in the late 19th century vitreous membrane

and the layer-fiber tissue, reminiscent of the corneal stroma formed during pathological processes, not related to damage of the eyeball (such as iridocyclitis). These observations have been questioned for a long time since it was believed that the formation of fibrous tissue in the anterior chamber must be a result of violation of the integrity of the corneoscleral zone to create conditions for the penetration of the stromal cells of the sclera and cornea in the eye cavity. Participation of the Descemet's epithelium in the formation of stromal tissue contrary to existing notions relative the histogenetic potency of this structure.

In the 60-70th of the last century by the works of Voyno-Yasenetsky V., Zimmerman L., Wolff S. it was proved that Descemet's epithelium is a real source of connective tissue (retrocorneal membrane) produced during inflammatory processes and burn without compromising the integrity of the eyeball. In these experimental researches it has proved the possibility of ectopic proliferation of the cells of posterior epithelium with formation of the films like the posterior membrane [5]. However, in these works, the focus was concentrated on the role of Descemet's epithelium in the formation of these films. In this discussion it is stayed by the fact. At the same time it remained out of the question the fact that these films can include not only the elements of Descemet's membrane, but also adjacent corneal stroma. So, it should be noted that during this process all these elements, including the epithelium of Descemet's membrane, the posterior membrane and posterior stromal layers are created with such structural precision that in some cases these films are preserving transparency without disrupting of eye vision. According to our histologic observations of these films the detectable stromal component usually does not exceed of 20-30 microns in its thickness. In other words, the thickness of stromal layer, that is reproduced by cells of the Descemet epithelium in pathological conditions, fits to the thickness of the layer, which describe H.Dua in the normal cornea.

It is also important to note that the given histomorphologic observation coincides with earlier embryological studies indicating on a two-stage formation of corneal stroma. Here we refer to the classical studies of eye's embryogenesis, which were held in the first half of the last century and has not yet been questioned [4]. Among these embryological studies should emphasize the transformation of the epidermal tissue in the corneal epithelium is carried out in the presence and close contact with the lens and the retina at the special role of cell-free substrate allocated from the optic cup. The presence of this substance is needed for induce epidermal epithelium aside corneal epithelium . Simultaneously, cells of mesenchyma, being introduced into the substrate, start to form collagen fibers and Bowman's membrane. Thus, the specific effect of the optic cup is the formation of a cell-free subepithelial layer, where the greater part of the cornea stroma is established.

Descemet epithelium begins to be formed later and has no inductive effects of noted above elements of the optic cup. Thus, during the formation of the corneal stroma, two distinct phases can be distinguished. On the one hand, this entrance of mesenchyma cells under subepithelial acellular layer and formation of collagen fibers under the epithelial lining. The second point is related to the Descemet's epithelium, which appears later in the form of a thin layer of the mesenchymal cells, that starts to form the germ of the corneal stroma near the periphery. The final formation of the corneal stroma (stromal fibers) requires the simultaneous presence of the anterior and Descemet's epithelium. Independent participation and influence of the two factors is also confirmed by the fact that the development of stromal tissue comes intense near Descemet's epithelium and endothelium, whereas in the center this process is slowed down [4].

Conclusions and prospects for further research

1. So, is there any reason to announce the opening of a new layer of the cornea? We believe that the issue of the sixth layer of the cornea appeared at the dawn of modern ophthalmology, and recent studies have, in fact, do not open, and summarize the discussion started over a hundred years ago.

2. Thus, long before the DALK problems there were facts pointing to the special status of the corneal stroma adjacent to Descemet's membrane. Morphological study of the process of regeneration and experimental embryology data show that the posterior section of the corneal stroma laying in the back of Descemet's membrane is a special layer having embryogenetic and histogenetic nuances and relationships that distinguish it from the rest corneal stromal tissue.

Researches mentioned above have added these facts. They not only identified topographical boundaries of the layer, but also showed that the embryo and histogenetic features of this part of the corneal stroma hide important nuances of its behavior in different clinical situations. In other words, private stroma of the posterior membrane of cornea, which deservedly can be called Dua's layer, is a real fact. The ophthalmic pathologists and ophthalmologists should be reckoned with this, regardless of whether it will be reflected in the official nomenclature and by what name.

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Салдан Й.Р., Маєвський О.Є., Салдан Ю.Й., Артемов А.В., Король А.П.
ШОСТИЙ ШАР РОГІВКИ: ВИГАДКА ЧИ РЕАЛЬНИЙ ФАКТ?

Резюме. *Існує безліч фактів, що вказують на існування окремої області строми рогівки, прилеглої до Десцеметової мембрани. Дані, отримані в процесі морфологічних досліджень регенерації рогівки, а також дані експериментальної ембріології вказують на те, що задня частина строми рогівки, розташованої над мембраною Десцемета, - це шар, що має особливості ембріо - і гістогенезу та відрізняється від основної строми рогівки.*

Ключові слова: *строма рогівки, Десцеметова мембрана.*

Салдан Й.Р., Маєвський А.Е., Салдан Ю.Й., Артемов А.В., Король А.П.
ШЕСТОЙ СЛОЙ РОГОВИЦЫ: ВЫМЫСЕЛ ИЛИ РЕАЛЬНОСТЬ?

Резюме. *Существует множество фактов, указывающих на существование отдельной области роговичной стромы, прилегающей к Десцеметовой мембране. Данные, полученные в процессе морфологических исследований регенерации роговицы, а также данные экспериментальной эмбриологии указывают на то, что задняя часть роговичной стромы, располагающейся над мембраной Десцемета - это слой, имеющий особенности эмбрио - и гистогенеза и отличающийся от основной стромы роговицы.*

Ключевые слова: *строма роговицы, Десцеметова мембрана.*

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