A PARALLEL BETWEEN THE BIOPHYSICS AND THE BIOMOLECULARITY OF THE CORNEAL TRANSPARENCY AND THE BIOMOLECULARITY IN THE OEDEMATOUS KERATOPATHY INDUCED BY THE CORNEAL ARCHITECTURE

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Abstract: *The basic principle of corneal transparency is primarily provided by the regularity of its components, thus minimizing the number of surfaces where the light can be refracted or reflected, this being the reason for which the biophysics of the corneal transparency is based on the stromal extracellular matrix. The main reason that leads to the loss of the corneal clarity is the disturbance of the regularity of its collagen fibres, the above-mention minimization being no longer able to take place in this case.*

The concordance between the anatomical and the physiological corneal factors, here in relation to the epithelial barrier, to the disposition of the corneal stroma lamellae, as well as to the endothelial barrier, contributes to the maintenance of the normal hydration of cornea; in its turn, this maintenance contributes to the keeping of its transparency. Once the electronic microscopy appeared, providing important data concerning the corneal ultra-structure, the interest concerning the study of its transparency increased, such transparency being an essential condition for a good visual acuity. Studies were carried out concerning the factors that impact the maintenance of corneal transparency, being well known that its depletion induces a quite high morbidity rate worldwide.

Biophysics of corneal transparency

Generally speaking, at the interface with the cornea, the light, which is a type of electromagnetic radiation with a wavelength that is visible for the human eye, may suffer the following phenomena: *1) its tract remains unaffected* - the deviation is maintained along the same axis with a fascicle of incident rays occurring along the optical axis at the interface between the light ray with the transparent cornea; *2) its reflection* - at the return of the light ray in its medium or when meeting the corneal surface; the reflection can be of the following types: *a) directed (regular):* when meeting the glazed surface, the reflected rays are parallel, just like the incident light rays and *b) diffuse (irregular):* when meeting the uneven separation surface, the reflection of the rays takes place in all directions; *3) its refraction* – when meeting the smooth interface of the transparent corneal medium that has different refractive indexes between the collagen and the surrounding basic substance, the light keeps its direction, under a different angle than the initial axis; *4) its absorption* - at the absorption of light through the optical media, when the light wave loses its energy by passing though the respective medium, the nature of the absorbing medium and the light wavelength are essential; *5) its scattering* – scattering (diffusion of the light) represents the physical phenomenon by which the light rays, invisible in a transparent medium, become visible provided that the concerned medium presents microscopic impurities or it is opaque. The explanation of this physical phenomenon of scattering lies in the production of secondary waves in the scattering medium, while their direction is different than the direction of the primary wave. For the case when the frequency of the secondary waves is the same as the frequency of the primary wave, the scattering is called elastic scattering (Rayleigh scattering), and when the frequency of the secondary waves is different than the frequency of the primary wave, the scattering is called inelastic.(1) The scattering takes place due to the presence of the non-homogeneities or of the density fluctuations that might appear in the media; due to the transfer of energy to the secondary waves, the intensity of the primary waves decreases. This light scattering includes: *a).random reflection*, where the particles suspended in the medium, due to their random disposition in relation to the incident light fascicle (these particles being at the same time large compared to the light wavelength), make the light be predominantly scattered back . *b).diffraction* – in which case the particles suspended in the medium, being relatively small for the light wavelength, make the light to be scattered in all directions; *c).diffuse refraction*, caused by the fluctuations of the refractive index along the medium, which make the light travel direction be deviated, but in the predominant initial direction.(2)

The two theories of the corneal transparency

The study of these theories was based on the light scattering from the extracellular matrix. The first theory refers to the fact that the light scattered through the individual fibres of the collagen network is annulled by the destructive interference from the light scattered at the level of other individual fibres (Maurice, 1957). The second theory of corneal transparency refers to the distance between the stromal collagen fibres that has to be less than 200 nm (Goldman and Benedek). Considering the difference between the refractive indexes of the collagen and of the surrounding basic substance that contains water, the proteoglycans and the glycosaminoglycans, that are 1.47 and 1.35 respectively, the corneal stroma should not be transparent, but it should be opaque, similar to the eye sclera, and even similar to the derma of the body.

The information obtained from specialized studies carried out along the years, concerning the transmission electronic microscopy, that cornea has a uniform and spaced arrangement of collagen fibres, led to the idea of differentiation from the collagen of other tissues of the body by its transparency. This very arrangement of the collagen corneal network represented the initial base for the first explanation of the corneal transparency proposed by Maurice, who took into consideration the theory of collagen network (the so

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called "lattice theory"), thus meaning to say that the small dimensions (32 nm) and the even spacing of the fibres in the network (64 nm) led to the lateral scattering of the light through the collagen fibres that were destructively disturbed based on the light scattering from the adjacent fibres that provide its transmission. Later on, Goldman ad Benedek brought a change to the theory of the network, taking into account the variations of the refractive index within the medium based on the distance, this second theory proposing that the light scattering in the medium depends on its wavelength and on the distance that separates the fluctuations of the refractive index and/or the extent of the fluctuations of the refractive index. To put it in other words, when the distance between the collagen fibres is smaller than half of the wavelength of the visible light (400-700 nm), the medium is transparent, while when the refractive index varies on distances that are longer than half of the wavelength, or 200 nm, significant light scattering appears.(3) These theories anticipate the turbidity of the pathological cornea, and in our case the oedematous cornea, in which case the corneal distribution of the collagen fibres is disturbed, due to the presence of numerous "lakes" of collagen fibres ("lattice"), areas where these fibres are absent.

To conclude, the minimization of light scattering by cornea is due to the fact that, at eye level, its characteristics that contribute to this thing are produced by the corneal epithelium, respectively by the fact that its inside and outside surfaces are smooth and even, and at the level of its own stromal substance, the collagen fibres are disposed in an even arrangement as well, this pertaining to their parallelism within each layer, compared to the texture unevenly disposed at the level of the sclera collagen, this also entailing the opacity of the latter. This minimization of the light scattering, which, according to Benedek, (4) is produced only by those fluctuations with a wavelength equal to or longer than half of the light wavelength in the concerned medium, provided by the uniformity of the distance between the stromal collagen fibres, is due to the water content of the basic substance which is and must be maintained within constant limits. In order to achieve this, the cuboidal cells that belong to the corneal endothelium, the most inner layer of cornea, activate the pump of ions and water from the basic corneal substance inside the aqueous humour, the effect being the prevention of the water excess, which, in its turn, is able to disturb the regularity of the collagen layers, thus causing the opacity of cornea, with repercussions for the visual acuity.

Biomolecularity of corneal transparency

The glycoproteins consist of several types of collagen (as these collagens are trimers, they have one, two or three distinct genes) and proline for the formation of alpha-helix. The glycine wraps these collagens in triple helix, thus presenting a triple helix field; they also contain two amino-acids, and hydroxyproline respectively, that has a role in the stabilization of the triple helix and the hydroxylysine that is important for the glycosylation. The glycosylation process has a determining role in the corneal transparency. The extracellular matrix of corneal stroma, that mainly consists of type I collagen and small amounts of type V collagen, also contains four proteoglycans, out of which three have keratan-sulphate chains: lumican, keratocan and mimecan (or osteoglycin) and one has a chondroitin-sulfate chain: decorin (dermatan-sulphate proteoglycor) the proportion being 50% for each category, the proteoglycans and the type V collagen regulating the growth of the collagen fibres. (5) Other components of cornea are type VI collagen that forms microfiber structures, Fibril Associated Collagens with Interrupted Triple Helices (FACIT) collagens (6) (types XII and XIV), and also non-fibrous collagens (types XIII and XVIII), and, following the experimental research, it was found that the keratan-sulfate proteoglycans act for maintaining the uniformity of the collagen fibre diameter, while the dermatan-sulfate proteoglycans have an essential role in maintenance of the inter-fibre spaces and of the adhesion of corneal collagen lamellae.(7)

Biomolecularity and corneal architecture affected within the induction of the oedematous keratopathy

The various architectural characteristics of the anterior and posterior corneal stromas, as well as the distribution of keratan-sulphate and dermatan-sulphate proteoglycans at the level of the two stromas, justify the different location of the corneal oedema, with the specification that it has a lower intensity from the posterior side towards the anterior side. This is explained by the cornea anatomical configuration, consisting of the orthogonal arrangement of the collagen fibres at the level of the posterior and medium stroma, in relation to the fact that from this medium level towards the anterior level, the collagen bundles gradually become wavy, curled and criss-crossed, this braiding ending in the formation of a cohesive structure in the most anterior 100-120 microns, where part of the anterior lamellae are unobtrusively inserted into Bowman layer, thus creating impermeability, resulting in the fact that the morphological modification at this anterior stromal level is not affected, even under the circumstances of the production of a major corneal oedema up to two-three times thicker than the initial thickness, while in the posterior stroma the collagen lamellae can be easily separated (all these being observed in histological studies). In the oedematous keratopathy, where the starting point is the impact on the corneal endothelium, the tendency of the corneal stroma is to become edematiated, having as a final outcome its opacification, the oedema especially occurring in the posterior-anterior direction, being thus the lowest in intensity in the furthest anterior side of cornea. This corneal opacity induced by the presence of the oedema as a result of a growth of corneal thickness, is explained by the increase of light scattering and the decrease of the corneal refractive index from 1.401 in the anterior surface (corneal epithelium) to 1,380 in the stroma and to 1.373 in the posterior surface.(7) Another explanation of the differentiated corneal oedema is justified by the different presence of the proteoglycans at the level of the anterior and posterior corneal stromas, here in relation to its presence in the posterior side of the keratan-sulphate that is a more hydrophilic proteoglycan, entailing its affinity for liquid, compared to the abundance in the anterior side of the dermatan-sulfate, a proteoglycan much less hydrophilic.

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