Archives of Ophthalmology and Optometry

Volume 1, Issue 1, 2018, PP: 28-30

What can Biophotonics tell us about the Structure and Physiology of the Cornea?

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KEYWORDS: collagen; corneal stroma; optical anisotropy; polarized light.

Biophotonics refers to the interplay of light with biological tissues [1]. Optical and infrared technologies based on the interaction of photons with tissues have been widely used to diagnostic and/or treat a variety of conditions in different ocular structures, including the cornea which is the major optical medium of the eye. Light interacts with ocular tissues in many diverse ways, depending on the energy or wavelength of the photon. In the cornea, light-tissue interactions have been routinely described using models proposed for crystal structures [2–7].

The cornea, like the crystals, is optically anisotropic, that is when interacting with polarized light it causes changes in the polarization state of the light [6, 8–10]. Interesting, there is no consensus over the form of anisotropy that cornea takes, probably because many authors had examined only the central area of the cornea [2–4]. For example, Stanworth and Naylor [5], based on interference figures called "isogyres" observed when excised corneal specimens were examined with a crossed polarizer-analyzer system, proposed that the human cornea behaves as a optically uniaxial crystal. In contrast, Van Blokland and Verheslt [7] proposed that the human cornea behaves like an optically biaxial crystal, showing the fastest axis being normal to its surface, and the slowest axis lying in the surface being generally nasally downward. Corneal anisotropy represents a hot topic among ophthalmologists and researchers in vision sciences because it stem from the same molecular lattice that determines transparency, refractive function or transmittance, and biomechanics of the cornea.

The source of optical anisotropy for the cornea is the supramolecular organization resulting from the highly ordered periodic arrangement of collagens fibrils/fibers into the stroma [8–10]. Compared with collagen fibrils in other structures of the body, those of the cornea are more hydrated and narrow, in addition to having a uniform diameter and being brought together to a high degree of lateral order [11, 12]. The crucial role of the stromal collagen features in controlling the corneal structure and physiology has long been known, since Maurice [13]. However, biophotonics/anisotropy has revealed new details of these features; consequently, new theories on the physical basis of the corneal transparency are also emerging, requiring that ophthalmologists and researchers keep an eye out for updates in the literature. An important finding revealed by the biophotonics is that the collagen dynamicity is governed by biocybernetic feedback mechanisms which generate spatiotemporal cellular signaling by intra- and intermolecular displacements and by piezoelectricity [14, 15]. In practical terms, the understanding that the corneal collagen can act as an "electric transducer" could be useful to develop strategies to improve corneal healing. Lasers or drugs that modulate collagen piezoelectricity, as well as wound-induced electric currents [16], can accelerate or reduce the rate of healing. Also, strategies that modulate collagen displacements and piezoelectricity can control the biosynthesis and extracellular mechanisms of self-assembly of this

molecule, inhibiting the progression of diseases that compromise corneal stability.

At present, the most accessible biophotonic tools for in vivo or ex vivo resolution of corneal structure and physiology are polarization-resolved second harmonic microscopy, polarization-sensitive spectral domain optical coherence tomography, polarized biomicroscopy, polarimetric interferometry, and polarized light microscopy. All of these devices explore the fact that when polarized light passes through the anisotropic sample, occurs changes in the amplitude and phase of the electric vector, generating optical effects known as dichroism and birefringence [6, 8–10]. Dichroism is still little explored in the ophthalmic practice. On the other hand, birefringence is part of the daily routine of ophthalmologists, reason by which to know details on the theoretical base of this phenomenon is fundamental to improve its clinical application.

Birefringence can be defined as the ability of the cornea to decompose the light into two orthogonally polarized rays with different velocities [8, 10]. The vectors experience a phase shift relative to each another, which is expressed by the equation: $\delta = (2\pi/\lambda) \times L\Delta_n$, where δ is the phase difference between propagating orthogonal polarizations, λ is the photon wavelength, L is the thickness of the sample, and Δ_n is the difference in the refractive indices between the two optical axes of the sample. $L\Delta_n$ is known as the optical path difference or retardation [8, 10].

Corneal total retardation is composed of two fractions, the intrinsic and the form birefringence [8]. The intrinsic birefringence has its origin in each collagen fibril and results from the asymmetrical alignment of chemical bonds or ions within the collagen particles [8, 17]. Unlike, the form birefringence is an anisotropy displayed by mixed asymmetric structures wherein rod-shaped molecules of a given refractive index are dispersed into preferential orientations, depending on the refractive index of the medium [17]. Collagen fibers are considered mixed structures because they result from a close association of collagen fibrils with non-collagenic molecules, especially proteoglycans, which regulate their diameter and degree of packaging [12, 18, 19]. In the case of the cornea, the form birefringence is a non-linear optical effect that results from the stack formed by the layers of stromal collagen fibers. Corneal form birefringence depends on the sub-wave dimension, geometry, and orientation of the rod-shaped triple chain collagen molecule, compatibility of the photon wavelength with the collagen molecule, and packaging and spatial organization of collagen fibrils/fibers [17].

Knowledge of the nature of intrinsic and form birefringence has many applications in the evaluation of corneal structure and physiology. Inevitably, changes in the supramolecular organization of the stromal fibers compromise the anisotropy of the cornea. Therefore, biophotonic monitoring of corneal birefringence is an effective strategy to establish how the cornea responds with physiological adaptation or remodeling to surgical interventions, chemical treatments, photoablation, mechanical stimuli, among others. Besides, knowledge of corneal anisotropy/birefringence is a need for the correct diagnosis of pathological conditions affecting other ocular structures. Failure to take corneal anisotropy into account may lead to erroneous results in procedures that employ polarized light as a diagnostic probe [2], including evaluation of the retinal nerve fiber layer by scanning laser polarimetry in glaucoma patients [20, 21] and polarimetric glucose monitoring in the aqueous humor of diabetic patients [22]. Assessment of birefringence through biophotonic tools has also helped in understanding the mechanisms by which the mammalian cornea, including humans, controls and maintains its curvature [23]. Under another perspective, anisotropy is also relevant for corneal bioengineering since it reports, at the nanoscale level, on the structural and molecular requirements needed to create an artificial functional stroma. Nowadays, 3D printing technology allows that an anisotropic supraorganization, such the cornea, to be reproduced in a laboratory.

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Citation: **Marcela Aldrovani, et al.** *What can Biophotonics tell us about the Structure and Physiology of the Cornea?. Archives of Ophthalmology and Optometry. 2018; 1(1): 28-30.*