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Experimental study on the mechanical strain of corneal collagen ☆

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ABSTRACT

Currently, investigations of biomechanical properties of the fibrous tunic are becoming even more topical, especially for diagnosis of corneal ectatic disease, as well as correct interpretation of intraocular pressure (IOP) parameters, particularly in patients with prior surgery on cornea. The study principle is based on the ability of substances to change optical anisotropy depending on mechanical strain applied to them. An experimental set-up was constructed which allows assessment of polarization degree of light which is emitted during luminescence of strained collagen. The study was performed on 18 corneoscleral discs of chinchilla rabbit eyes at 15 and 50 mm Hg pressure, among them in 6 cases before and after making radial incisions, and in 6 cases before and after conducting the mechanical cornea abrasions that were asymmetrical by depth until reaching the local zone of iatrogenic keratectasia. Corneal collagen mechanical strain mappings were formed on 3 experimental models (intact cornea, cornea post radial keratotomy and keratectasia) under intra-chamber pressure of 15 and 50 mm Hg. Corneal collagen mechanical strain is evenly allocated in the intact cornea. After radial keratotomy the main mechanical loading was concentrated over the middle part of corneal periphery, particularly in the bottom of keratotomic incisions. The increased intra-chamber pressure made the strain rise in those models. Upon cornea abrasion the main straining is distributed within the thinning zone, and the increase of intra-chamber pressure only increases the load over residual stroma. A new principle of corneal biomechanical properties investigation based on assessment of degree of light polarization emitted during luminescence of strained collagen, has been proposed and experimentally tested.

1. Introduction

Currently, investigations of biomechanical properties of the fibrous tunic are becoming even more topical, especially for diagnosis of corneal ectatic disease, as well as correct interpretation of intraocular pressure (IOP) parameters, particularly in patients with prior surgery on cornea (Fabian et al., 2011; Pepose et al., 2007; Qazi et al., 2009).

Keratoconus is one of the most widespread ectatic corneal disorders. It is a chronic progressive disease characterized by biomechanical impairment of the cornea, change of its surface profile and decrease of visual acuity. Nowadays early diagnostics of this pathology is complicated (Saad et al., 2010; Schweitzer et al., 2010).

In keratoconus there is a local plastic instability of the cornea in its central part, which then transforms into an ectasia. In this connection a method of mapping the irregular distribution of mechanical strains in the cornea and detection of "weak" zones for early diagnostics of keratoconus should be developed.

The method of mechanical strain visualization in cornea is based on assessment of substance optical anisotropy. Different authors studied anisotropy *in vivo* using transparent achromatic light (Bueno and Vargas-Martin, 2002; Bour, 1991), whereas polariscopic luminescent imaging of stromal collagen can provide more detailed mapping of mechanical strain in the cornea.

2. Background of using polariscopic luminescent imaging for biomechanical status of stromal collagen

Luminescent emission of some objects is polarized. Polarization can be complete or partial, linear or circular resulting from anisotropy of absorption and emission elementary events of luminescence. Primary anisotropy of the medium implies unidirectional orientation of most atomic, ionic and molecular structures. Induced anisotropy of the medium can be determined by the emitter orientation in the external field (electric, magnetic, mechanical) or excitation anisotropy (esp. when polarized light is used for excitation) (Steinberg, 1978).

The degree of system anisotropy is a quantitative characteristic of polarization. Formula (1):

$$\Delta n = (I_1 - I_2)/(I_1 + 2I_2)$$

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where I_1 and I_2—the intensities of mutually perpendicular polarized components of luminescence. In anisotropic medium these are maximal and minimal components; in isotropic medium they correspond to components polarized parallel and perpendicularly to electric field vector.

For linearly polarized light $\Delta n=1$, for nonpolarized $\Delta n=0$.

Studies of polarized luminescence provide information on composition of elementary emitters (atoms and molecules of substances in different aggregate states) and on interaction between emitters and between emitters and the medium.

Observing cornea luminescence we deal with a weakly ordered heterogeneous optical object with partially oriented molecules.

In mediums like this we can define two parts: fully oriented and chaotic ones. The first part of the medium can emit polarized luminescence even at isotropic excitation (spontaneous polarization). Polarized luminescence of the other part is only possible at anisotropic excitation. Studying polarized luminescence of such mediums allows us to evaluate the degree of their order, dynamics and orientational behavior of the emitting particles.

Among mediums with partially oriented particles we can name films and macromolecule fibers (polymer, liquid-crystal and others) and biological objects. Studying rotational depolarization of luminescence provides data on intramolecular mobility and integrated movements of macromolecules, i.e. intra- and intermolecular interactions, protein conformations, intracellular plasma viscosity, functioning of biologically active substances, mechanisms of muscle fiber contraction, structure of biological membranes, etc. Effects of luminescent polarization are well-known and have been studied both on polymers and on organic subjects like heme (Gussakovsky et al., 2000; Lakhwani et al., 2009).

Application of additional anisotropic mechanical force to such a complex heterogeneous system improves the degree of its order and intensifies luminescence polarization.

The improvement of the degree of system order implies:

1. reposition of luminescent proteins at tissue deformation (Fig. 1);
2. appearance of a dominating orientation of reflecting surfaces (Fig. 2);
3. magnification of anisotropic tension of intermolecular interaction in intracellular liquid (Fig. 3);

Thus, those areas of stroma optical medium that experience greater mechanical deformation show a higher level of anisotropy leading to highly polarized luminescence.

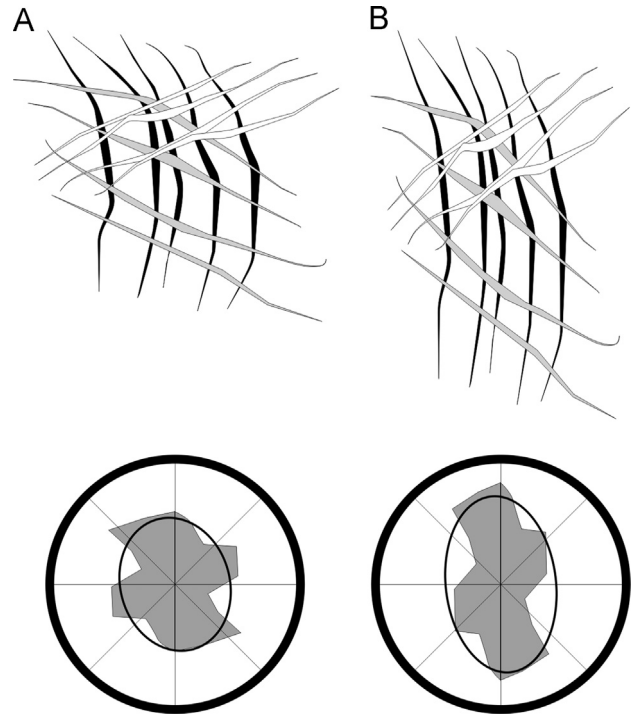

Fig. 1. Arbitrary orientation of structural groups of collagen molecules without any applied force (A) and at deformation (B). Rose-diagrams of spatial orientation of collagen structural elements and theoretical two-dimensional indicatrix of protein system luminescence polarization are shown below.

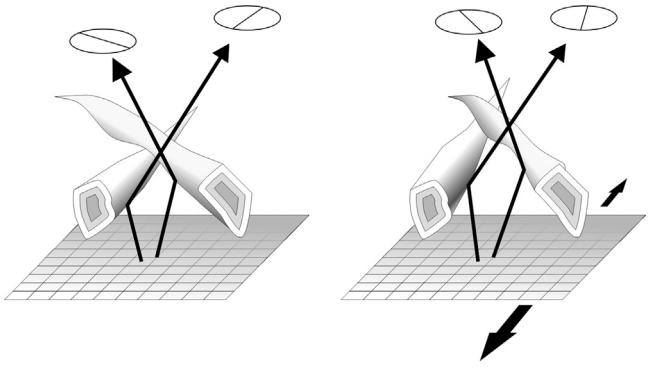

Fig. 2. Change of polarization vector of the light beam reflected from optical interface at reposition of corneal structural elements induced by applied mechanical force.

3. Material and methods

A chamber was constructed which allowed experimental polariscopic examination of corneoscleral disc and simulation of various IOPs. One end of a hollow cylinder (aluminum alloy) is covered with airtight quartz glass and the other end is a ring clamp which is shaped to fit the form of a rabbit eye. The chamber is connected to a manometer via a pipe. The pressure is applied with a manual pump.

The collagen luminescence excitation was initiated using two luminescent lamps with maxima at 390, 400 and 415 nm. Digital photo registration was carried out through the dichroic filter with 99% cutoff of excitation radiation, and rotating linear polarizer. The polarizer was rotated with 15° intervals, and photo registration was carried out every time. A series of 12 images was formed at each polarizer position.

From the series, pairs of images were selected which were obtained at a mutually perpendicular positions of polarizing filter's polarization planes. For every pair point the absolute values of luminescence brightness difference (ΔB) were calculated using the formula (2):

$$\Delta B^{\alpha}_{(x,y)} = |B^{\alpha}_{(x,y)} B^{\alpha+90}_{(x,y)}|,$$

where: $\Delta B^{\alpha}_{(x,y)}$ is the absolute value of the tissue luminescent brightness difference in the point with (x,y) coordinates for a pair of images obtained at a mutually perpendicular position of polarizing filter's polarization planes, $B^{\alpha}_{(x,y)}$ is the brightness of the point with (x,y) coordinates of the first image in the pair, $B^{\alpha+90}_{(x,y)}$ is the brightness of the point with (x,y) coordinates of the second image in the pair, and α is the angle of polarization plane for the first image in the pair.

The processed data is written into a two-dimensional array. Considering that the excitation radiation intensity (fluorescent lamps in the experiment area produce illumination of approximately 340 lx) and other conditions of the photo registration (optical exposure, lens diaphragm, image sensor magnification) are constant, acquired small values are multiplied by a static coefficient for the purpose of visualization. The coefficient is chosen empirically ($K_{corr}=3.2\times10^3$) with the idea of keeping the digital brightness of a resulting mapping in the 0–255 range excluding artifacts. Static normalization enables visual comparison of the acquired mappings.

The degree of optical polarization for every point in the series of images was evaluated using the formula (3):

$$\Delta n'_{(x,y)} = \frac{\sum_{\alpha=0}^{180} \Delta B^{\alpha}_{(x,y)}}{\sum_{\alpha=0}^{180} B^{\alpha}_{(x,y)}},$$

where: $\Delta n'_{(x,y)}$ is the degree of optical polarization in the point with (x,y) coordinates; $\Delta B^{\alpha}_{(x,y)}$ is the absolute value of the tissue luminescent brightness difference in the point with (x,y) coordinates for a pair of images obtained at a mutually perpendicular position of polarizing filter's polarization planes, and at the first image polarization plane position under the α angle; $B^{\alpha}_{(x,y)}$ is the brightness of