EC OPHTHALMOLOGY
Research Article

The Morphological and Biochemical Changes in the Sclera after Exposure to the Laser Diode. Experimental Study

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Abstract

Purpose: To investigate the morphological and biomechanical changes in the sclera after exposure to diode laser in the experiment.

Materials and Methods: The study included 12 isolated fragments of human cadaver sclera of 12 eyes aged 60 to 70 years. [Axial length (AL) is 23.56 ± 3.06 mm (20.05 - 26.62)]. Exclusion criteria were: eye injuries, surgical interventions before, as well as the general anamnesis of medical illness. Samples of sclera were cut out with a microsurgical blade from the posterior pole of the eye, then the scleral fragments were exposed to the diode laser ALOD-1 (Russia) (energy E = 900 mJ, exposure time 5 sec). Fragments of the sclera treated earlier by the laser were cultivated in the medium and then transported to a laboratory for biomechanical testing on an Instron testing machine, and morphological studies using electron microscope Quanta 200 3D.

Results: The study of biomechanical properties of posterior pole of sclera after the exposure to diode laser showed an increase in strength and indices Jung's modulus (JM) in all tissue samples regardless of the refraction of the eye. The morphological studies using the electron microscope Quanta 200 3D. Diode laser exposure leads to pronounced morphological changes in the scleral tissue.

Conclusion: Strength properties of samples of scleral tissue after the exposure to the diode laser increased by 30% in all persons (regardless of the refraction of the eye), which may be relevant for the development of new methods in treatment of glaucomatous optic neuropathy.

The lowest biomechanical characteristics are detected in the fragments of the myopic type sclera that causes pronounced changes in the biomechanical status initially in comparison with other types of refraction.

Modeling of the biomechanical properties (JM, strength) in the most prognostically dangerous areas (the posterior pole of the eye), enables a future development of a method of invasive impact on the fibrous membrane of the eye in open-angle glaucoma.

Keywords: *Sclera; Diode Laser; Biomechanical Parameters; Morphology*

Introduction

Despite of the existing successes achieved in the early diagnosis and treatment of the glaucoma, the proportion of patients with glaucoma is steadily increasing [12]. It is suggested that changes in the elasticity and elasticity of the scleral membrane may be one of the key factors in the development of the glaucoma. Most surgical interventions in terminal of glaucoma are traumatic and are aimed at correcting the hydrodynamics and rigidity of the scleral membrane of the eye [14,17,19]. According to O. V. Svetlova., *et al*. (2003) a decrease in the rigidity of the scleral shell of the eye can occur due to changes in the outer highly rigid layers of the sclera and the effective inclusion of its deeper layers, which have not lost their elastic-plastic properties [10,11,15-17].

Contact transcleral diode-laser cyclophotocoagulation (CTDCC) has been widely used in recent years as a method that allows not only to reduce ophthalmotonus, but also to stop pain syndrome [2-4]. However, biomechanical and morphological studies of the fibrous membrane of the eye after exposure to a diode laser do not lose their relevance at the moment.

Biomechanical models being developed today are very useful for developing methods for the prevention and treatment of various eye diseases, therefore, the study of the biomechanical properties of the sclera will allow us to create and develop new methods for the treatment of the glaucomatous opticoneuropathy [21].

Purpose of the Study

To investigate morphological and biomechanical changes in the sclera after exposure to a diode laser with a wavelength of 810 nm in an experiment.

Materials and Methods

12 isolated fragments of the sclera of 12 human cadaver eyes were studied; age - from 60 to 70 years old, 4 men, 2 women. The average value of the anteroposterior axis (PZO) of the cadaver eyes is 23.56 ± 3.06 mm (20.05 - 26.62), the measurement was carried out using an electronic caliper (Heyco, Germany). Based on the PZO data, all cadaver eyes were divided into groups according to the type of axial refraction of the eyes. With hypermetropic type of the refraction - 4 eyes, with myopic type of the refraction - 4 eyes, with emmetropic type of the refraction - 4 eyes. The selection criteria for cadaver eyes were the highest atropine test and the sampling period of the material up to 14 hours.

The exclusion criteria were: eyes with injuries, previous surgical interventions, a history of common connective tissue diseases.

Scleral specimens with a standard width of 5 mm. were cut out with a microsurgical blade from the posterior pole of the eye, after which they were exposed to an ALOD-1 diode laser (Russia) with a wavelength of 810 nm. (energy E = 900 mJ, exposure 5s.) The control group consisted of samples of the sclera of the same eyes without making any impact of them.

Then the scleral fragments were cultured in a medium containing 96% DMEM/F12, 3% fetal bovine serum, and 1% antibiotics. The cultivation period was 14 days, with a medium change every 2 days. The research was carried out with the support of the Center for Fundamental and Applied Biomedical Problems of the Federal State Autonomous Institution "MNTK" Eye Microsurgery "named after acad. S.N. Fedorov.

Biomechanical tests of scleral specimens were carried out in a single loading mode, up to rupture, on an Instron -3322 universal testing machine. The curve of the dependence of the specimen elongation on the applied load was recorded on the monitor screen, after which the corresponding elastic-strength parameters of the sclera were calculated. The studies were carried out in the laboratory of strength and plasticity of metal and composite materials and nanomaterials No. 10 of the FSBI IMET RAS named after A.A. Baikov.

After biomechanical testing, scleral tissue was dipped in 10% formalin solution and examined using a Quanta 200 3D scanning ion-electron microscope. The research was carried out at FGBU NIIEM them. N.F. Gamalei.

Using a Quanta 200 3D scanning electron microscope, an analysis of the scleral tissue morphology after laser exposure was carried out. For scanning electron microscopy, scleral tissue samples were plated with gold up to 1 mm at P = 10^{-2}ATM.

Results

In the study of the biomechanical properties of fragments of the sclera of the posterior pole of the eye after diode-laser exposure, an increase in strength and Young's Modulus was noted. When compared with the control group, the strength indicators of scleral tissue after exposure to a diode laser significantly ($p < 0.05$) increased and amounted to 17.3 ± 1.1 MPa in individuals with a hyperopic type of axial refraction, 13.6 ± 1.8 MPa - with myopic type of axial refraction, 17.1 ± 1.4 MPa - with emmetropic type (Table 1).

	Hypermetropia	Emmetropia	Myopia
After laser	17,3 ± 1,1*	17,1 ± 1,4	13,6 ± 1,8*
Control	13,6 ± 0,42*	12,7 ± 1,2	10,1 ± 1,2*

Table 1: *Scleral strength, MPa (M ± σ) after laser exposure on eyes with different types of axial refraction.*
**: Differences between norm, myopia and hyperopia are statistically significant P ≤ 0.05.*

The value of Young's Modulus significantly ($p < 0.05$) increased in sclera samples previously exposed to the laser and amounted to 49.1 ± 2.1 MPa in individuals with hypermetropic axial refraction type, 32.2 ± 2.8 MPa - with axial myopic type refraction, 48.9 ± 1.5 MPa - with emmetropic refraction (Table 2).

	Hypermetropia	Emmetropia	Myopia
After laser	49,1 ± 2,1*	48,9 ± 1,5	32,2 ± 2,8*
Control	37,3 ± 2,4*	36,8 ± 1,6	24,1 ± 3,2*

Table 2: *Young's Modulus, MPa (M ± σ) after laser exposure on eyes with different types of axial refraction.*
**: Differences between norm, myopia and hypermetropia are statistically significant P ≤ 0.05.*

Strength and MY values increase in all scleral specimens, regardless of the type of axial refraction. Fragments of the sclera with a myopic type of axial refraction have the least strength characteristics, which causes changes in the pathogenetic status initially.

When carrying out scanning electron microscopy of cultured sclera samples after laser exposure, morphological changes in the sclera were revealed in comparison with the control group (Figure 1-3).

The most pronounced morphological changes in the sclera were found in individuals with myopic type of axial refraction, manifested in particular: thickening of collagen fibers, chaotic arrangement in space, an increase in the distance between bundles of collagen fibrils and the formation of thin collagen structures of a cobweb-like consistency between the fibers (Figure 1 and 2).

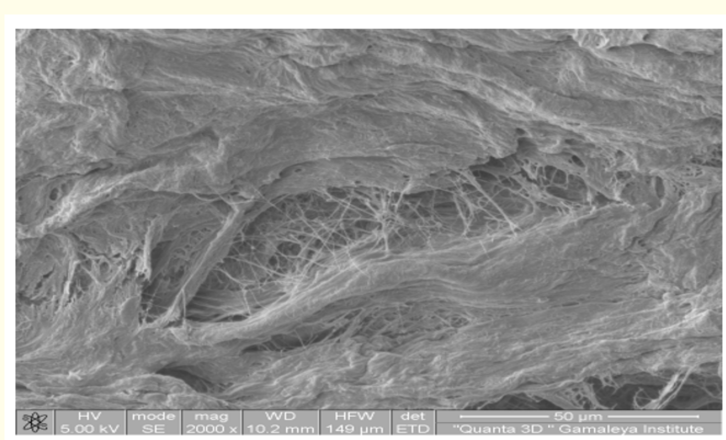

Figure 1: *Sclera after exposure to a diode laser in persons with myopic type of axial refraction. Magnification 1000.*

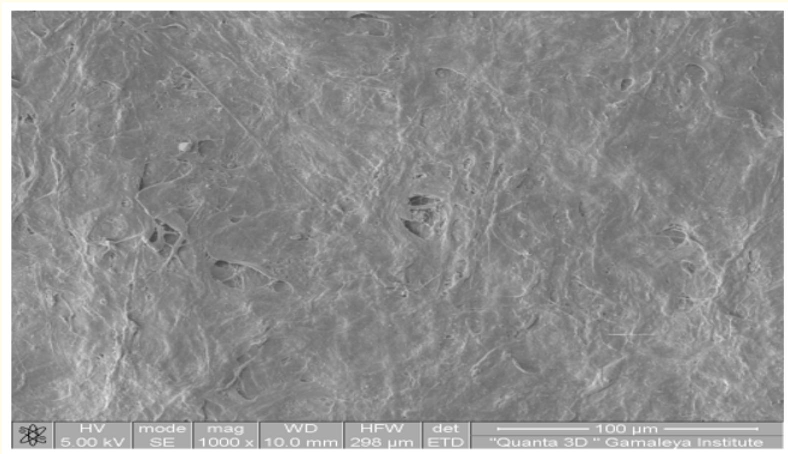

Figure 2: *Sclera of the control group in persons with myopic type of axial refraction. Magnification 1000.*

When carrying out scanning electron microscopy of cultured sclera samples after laser exposure in individuals with hypermetropic and emmetropic types of axial refraction, morphological changes in the sclera were found, when compared with the control group (Figure 3-6).

Morphological changes in the sclera were manifested in the thickening of collagen structures, an increase in the distance between bundles of collagen fibrils, and the formation of single thin cobweb-like collagen structures between the fibers.

Discussion

The study of the biomechanical properties of the sclera of the posterior pole of the eye after exposure to a diode laser showed an increase in strength and Young' Modulus in all sclera samples, regardless of eye refraction. The results of obtained demonstrate a high correlation between strength and Young's Modulus (MY) in all groups.

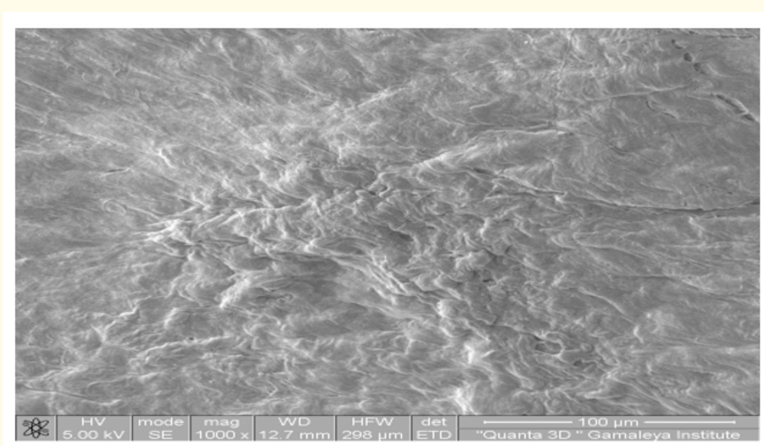

Figure 3: *Sclera after exposure to a diode laser in persons with hypermetropic axial refraction. Magnification 1000.*

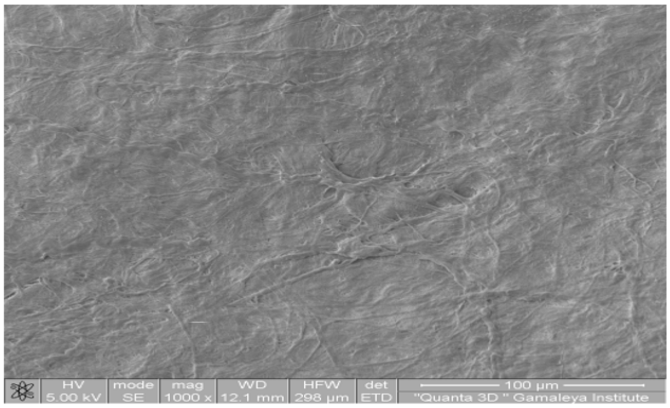

Figure 4: *Sclera of the control group in persons with hypermetropic axial refraction. Magnification 1000.*

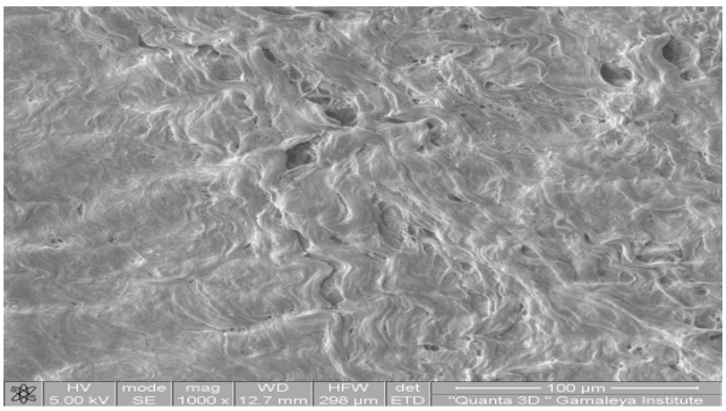

Figure 5: *Sclera after exposure to a diode laser in persons with emmetropic axial refraction. Magnification 1000.*

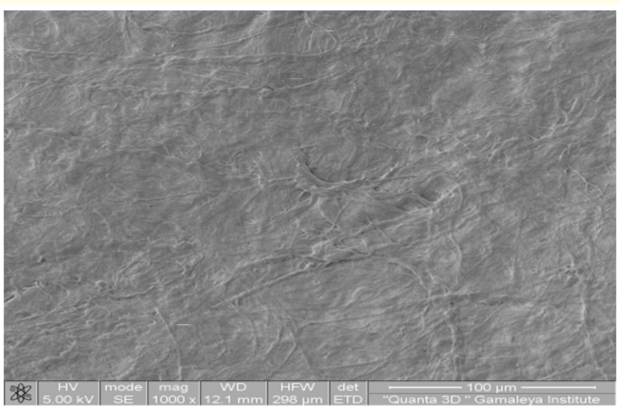

Figure 6: *Sclera of the control group, in persons with emmetropic type of axial refraction. Magnification 1000.*

Involutional changes in the sclera at the morphological level are reduced to a denser packing of fibrils, a thickening of the main cementing substance, which a qualitative and quantitative redistribution of glycosaminoglycans (GAG) occurs, however, after exposure to a diode laser, thickening of collagen fibers is observed, an increase in the distance between bundles of collagen fibrils and the formation thin cobweb-like collagen structures between the fibers, as a result of which the biomechanical parameters of the sclera change.

According to the literature, most surgical interventions are aimed at correcting the hydrodynamics and rigidity of the eyeball in advanced and terminal stages of the glaucoma, are traumatic and require a long postoperative period of treatment. At the moment, the ophthalmological society is looking for new, low-traumatic methods of treating the glaucoma and glaucoma optics neuropathy. Taking into account the changes in biomechanical properties, the data can be used to construct a mathematical model of the fibrous membrane of the eye and will allow in the future to develop new pathogenetically directed methods of treating the glaucoma optic neuropathy.

Conclusion

1. The strength indices of scleral tissue samples after exposure to a diode laser increase by 30% in all individuals, regardless of the refraction of the eye, which may be importance in the development of new methods of treating the glaucoma optic neuropathy.
2. Fragments of the sclera with the myopic type of refraction have the least biomechanical characteristics, which causes pronounced changes in the biomechanical status initially in comparison with other types of refraction.
3. Diode laser action leads to pronounced morphological changes in the sclera, which at the biomechanical level is accompanied by an increase in the strength characteristics of the tissue.

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