Femtosecond Corneal Collagen Crosslinking in Treatment of Patients with Progressive Keratoconus Stages I-II

DOI: 10.17691/stm2016.8.1.17
Received October 23, 2015

©

K.B. Letnikova, PhD Student, Department of Retina and Optic Nerve Pathology;
A.T. Khandjan, MD, PhD, Researcher, Department of Retina and Optic Nerve Pathology;
O.G. Oganesyan, MD, DSc, Senior Researcher, Department of Traumatology and Reconstructive Surgery;
A.V. Penkina, MD, PhD, Ophthalmologist, Department of Pathology of Refraction, Binocular Vision and Ophthalmoergonomics;
V.V. Neroyev, MD, DSc, Professor, Director

Moscow Helmholtz Research Institute of Eye Diseases, 14/19 Sadovaya-Chernogryazskaya St., Moscow, 105062, Russian Federation

The aim of the investigation was to assess safety and efficacy of corneal collagen crosslinking technique using femtosecond laser for intrastromal pocket creation in patients with progressive keratoconus.

Materials and Methods. Thirty-three patients (34 eyes) with progressive keratoconus stages I–II underwent femto-crosslinking of the corneal collagen. FEMTO LDV Z6 femtosecond laser was used to create an intrastromal pocket of 8.0 mm diameter and 140 µm depth. Normotonic riboflavin solution (0.1% riboflavin and 20% dextran) was instilled into the created pocket every 2 min during 30 min (15 instillations). At the same time UV-irradiation was performed using OPTO-XLink system (OPTO, Brasil) with 365 nm wavelength and an irradiance of 3.0 mW/cm^2 (5.4 J/cm^2). Changes in uncorrected visual acuity, corneal biomechanical properties, keratometric, topographic, pachymetric and morphological data were evaluated. The follow-up period amounted to 6 months.

Results. Preoperative visual acuity level recovered on day 3–5. Six months after the procedure the mean value of astigmatism decreased from 4.35±0.19 to 3.75±0.20 dioptres ($p<0.05$). The corneal thickness in the area of keratoectasia descended from 437.50±2.84 to 405.0±2.99 µm ($p<0.05$). Maximal corneal refractive power decreased from 52.15±0.23 to 51.05±0.21 dioptres ($p<0.05$). Significant elevation of corneal resistance (from 7.70±0.12 to 8.25±0.10 mm Hg) and corneal hysteresis indexes (from 6.90±0.10 to 7.30±0.11 mm Hg) was reported. Confocal microscopy showed increase in extracellular matrix density without the signs of edema, and corrugation due to cross-linkage effect. Regeneration of subepithelial and stromal nerve fibers was visualized. The posterior stroma and endothelium remained intact. Exposure to UV-crosslinking with riboflavin was limited to the anterior and middle corneal stroma (to 320–330 µm) and did not involve the posterior stroma and the endothelium. Six months after the operation OCT examination confirmed the existence of the demarcation line which lay at the depth of 320–330 µm.

Conclusion. Corneal collagen crosslinking with creation of an intrastromal pocket using a femtosecond laser appears to be an effective technique for keratoconus progression stabilization. The epithelial layer preservation in femtosecond crosslinking makes it possible to lower the risks of infectious complications of the eroded corneal surface, to reduce the patients' visual discomfort and pain in the early postoperative period providing a faster recovery.

Key words: femtosecond crosslinking; progressive keratoconus; corneal collagen crosslinking.

Keratoconus is a non-inflammatory degenerative bilateral disorder of a multifactor background accompanied by deformation, thinning and reduced biomechanical stability of the cornea [1]. In the early stages of this pathology visual acuity lowers due to myopia and irregular astigmatism. As a rule, classic keratoconus starts and progresses at puberty and tends to stabilize in patients after the age of 40, though the disease onset has been reported to occur at 30–50 years [2]. Progressive character of the disease can lead to significant visual impairment and, consequently, low quality of life.

Corneal collagen crosslinking is a surgical procedure performed to halt keratoconus progression [3]. The currently employed corneal collagen crosslinking technique was developed by Wollensak, Spoerl, Seiler at the University of Dresden in the late 1990s and was called “the standard protocol” [4]. This method is the photopolymerization of stromal fibers resulting from the combined effect of riboflavin and ultraviolet (UV) radiation exposure on the cornea. Consequently, additional bonds are formed between the corneal collagen molecules, its resistance to endogenic protease (which is a probable cause of ectasia development) is enhanced, mechanical rigidity of the cornea increases, keratoconus progression stabilizes, the need for corneal transplantation is prevented [5]. When corneal collagen crosslinking is performed in a standard way the epithelium in the optical area is mechanically removed, riboflavin solution is instilled and the cornea is subsequently exposed to

For contacts: Kseniya B. Letnikova, letnikova@inbox.ru

UV-radiation. However, epithelial debridement results in painful early postoperative period, slow healing and the risk of developing infectious keratitis [6]. There are different techniques used to improve riboflavin penetration through the intact epithelium [7, 8]. One of them is femtosecond crosslinking introduced by ophthalmologist John Kannelopulos in 2007. In fact, this technique involves using a femtosecond laser to create an intrastromal pocket in the cornea where riboflavin solution is later introduced, and the cornea is subsequently irradiated with UV light [9].

Femtosecond laser offers the possiblity to control the size and depth of the corneal pocket position thus making the operation technically easier, safer and more predictable. Ultrashort pulses of a femtosecond laser allow surgeons to create high power density of nearly 1,010 mW/cm^2 and more in the optical system focus, which enables surgeons to perform a super-accurate incision of the living tissue without its collateral damage [10]. Optical break-down underlying plasma-induced ablation and photodisruption occurs in the irradiated tissue. In this case the probability of thermal damage to the tissue is exceptionally low due to ultrashort pulses. Infrared femtosecond laser radiation easily penetrates through optically transparent media, so it can be focused at any point either on the surface or inside the tissue [11].

Undeniable advantages of femtosecond crosslinking include selective access of riboflavin into the anterior stroma, leaving the corneal epithelium layer intact, minimizing the risk of infectious complications, less painful healing of the cornea and, as a result, a shorter period of patients' rehabilitation [12, 13].

The aim of the investigation was to assess safety and efficacy of corneal collagen crosslinking technique with creation of an intrastromal pocket using a femtosecond laser in patients with progressive keratoconus stages I–II.

Materials and Methods. Thirty-three patients (34 eyes) aged 16 to 35 years with progressive keratoconus stages I–II underwent corneal collagen crosslinking at the Moscow Helmholtz Scientific Research Institute of Eye Diseases (Russia). FEMTO LDV Z6 (Ziemer, Switzerland) femtosecond laser was used to create an intrastromal pocket. The study enrolled the patients in early stages of the disease whose ectasia progression was proved by clinical, instrumental and diagnostic findings in dynamics. Increase in the maximum corneal refractive power (Kmax) by 1 diopter and more, revealed by the Galilei G6 Sheimpflug analyser (Ziemer, Switzerland), during 6–12 months was a criterion of degenerative process progression. The study included patients without corneal opacity and visual acuity with maximum correction of 0.4 and more. The study complies with the Declaration of Helsinki (adopted in June 1964 (Helsinki, Finland) and revised in October 2000 (Edinburgh, Scotland)) and was performed following approval by the Ethics Committee of the Moscow Helmholtz Scientific Research Institute of Eye Diseases. Written informed consent was obtained from every patient.

Along with the standard ophthalmological examination, before and after the operation the patients underwent examination with the Galilei G6 Sheimpflug analyser, evaluation of biomechanical properties of the cornea with the ORA device (Reichert Inc., USA), confocal microscopy using Confoscan 4 device (Nidek, Japan) and optical coherence tomography using Visante OCT (Carl Zeiss, Germany). All examinations were performed before the operation, 1 week, 3 and 6 months after the treatment (See the Table). The study included no patients with corneal inflammatory diseases, marked dry-eye syndrome and pregnant women.

An intrastromal pocket of 8.0 mm diameter and 140 µm depth (the access tunnel width and depth were 3.0 and 5.04 mm) was created using FEMTO LDV Z6 femtosecond laser under locally instilled anesthesia. Normotonic riboflavin solution (0.1% riboflavin and 20% dextran) was injected into the created pocket (Figure 1) with a blunt-ended cannula 1–2 instillations every 2 min during 30 min (15 instillations). UV-irradiation was performed using OPTO-XLink system (OPTO, Brasil) with 365 nm wavelength and an irradiance of 3.0 mW/cm^2 (5.4 J/cm^2). UV light exposure continued for 30 min simultaneously with riboflavin instillations. On completing the procedure antibacterial preparation was instilled, a soft contact lens was applied.

In the postoperative period the patients were administered instillations of antibacterial preparations,

The results of studying clinical and functional indexes after corneal collagen femto-crosslinking

Parameters	Before the operation	After the operation		
		1 week	3 months	6 months
Cylinderical component (dioptres)	4.35 ±0.19	4.85±0.19	3.97±0.18	3.75±0.20*
Corneal thickness (µm)	437.50±2.84	415.25±3.33*	410.61±3.01*	405.0±2.99*
Corneal refractive power (dioptres)	52.15±0.23	53.15±0.19*	52.13±0.21	51.05±0.21*
Corneal resistance factor (mm Hg)	7.70±0.12	8.10±0.11*	8.21±0.11*	8.25±0.10*
Corneal hysteresis (mm Hg)	6.90±0.10	7.12±0.11	7.24±0.12*	7.30±0.11*

* $p<0.05$ (comparison was carried out according to Student's t-test) — statistically significant difference in values compared to the indexes before the treatment.

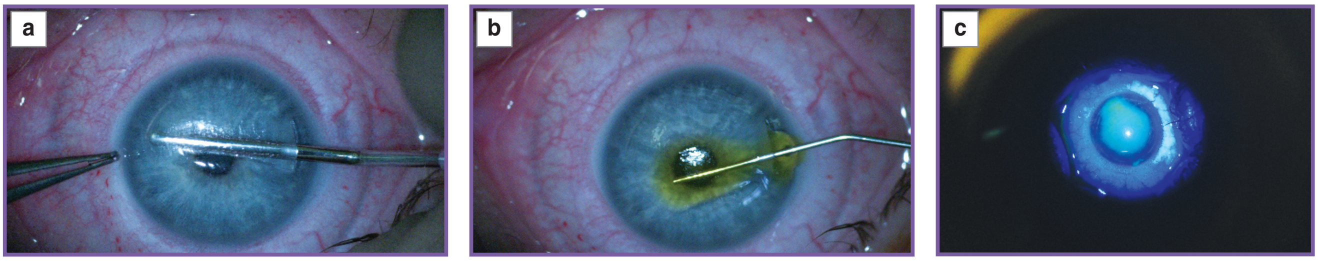

Figure 1. Stages of femtosecond crosslinking before UV irradiation: (a) dissection of the intrastromal corneal pocket created with a femtosecond laser; (b) riboflavin introduction into the intrastromal corneal pocket; (c) yellow glow of riboflavin located in the intrastromal corneal pocket, in a blue cobaltous filter

corticosteroids, tear substitution and reparative therapy was conducted. No postoperative complications were observed.

Results and Discussion. Initial corrected visual acuity amounted to 0.5±0.02. Preoperative corneal thickness was 437.5±2.84 µm. The studied group included patients with Kmax 52.15±0.23 dioptres. When creating an intrastromal pocket with a femtosecond laser no intraoperative complications were observed. During the first 5–6 h after the operation the patients complained of moderate pain in the operated eye, tearing, photophobia. Therapeutic contact lenses were removed 1 day after the operation. On biomicroscopy of the cornea 1 week after the operation a mild stromal edema was observed, the demarcation line was visualized in the corneal stroma.

Preoperative visual acuity level restored on day 3–5. No postoperative complications were observed. Six months after the procedure the mean value of corneal astigmatism decreased from 4.35±0.19 to 3.75±0.20 dioptres ($p<0.05$). Corneal thickness in keratoectatic area descended from 437.50±2.84 to 405.0±2.99 µm ($p<0.05$). Kmax value decreased from 52.15±0.23 to 51.05±0.21 dioptres ($p<0.05$) (Figure 2).

Statistically significant elevation of corneal resistance indexes (from 7.70±0.12 to 8.25±0.10 mm Hg) was reported. Corneal hysteresis increased from 6.90±0.10 to 7.30±0.11 mm Hg ($p<0.05$).

Six months after crosslinking isolated hyper-reflective epitheliocytes were visualized (up to 10 per field of vision). Polymorphism of basal epithelium became less expressed, cell boundaries remained well-defined. Increase in extracellular matrix density without the signs of edema was observed. There was corrugation due to cross-linkage effect. Regeneration of subepithelial and stromal nerve fibers was visualized. The posterior stroma and the endothelium remained intact (Figure 3).

The demarcation line could be observed during optical coherence tomography examination six months after the operation at the depth of 320–330 µm (Figure 4).

Thus, corneal collagen crosslinking with creation of an intrastromal pocket using a femtosecond laser appears to be an effective technique for keratoconus progression stabilization. The epithelial layer preservation in femtosecond crosslinking permits to lower the risks of infectious complications of the eroded corneal surface, to reduce the patients' visual discomfort and pain in the early postoperative period providing a faster recovery. The advantages of crosslinking with creation of an intrastromal pocket using a femtosecond laser include fast penetration of riboflavin and its high concentration in the stroma, which ensures well-marked and profound

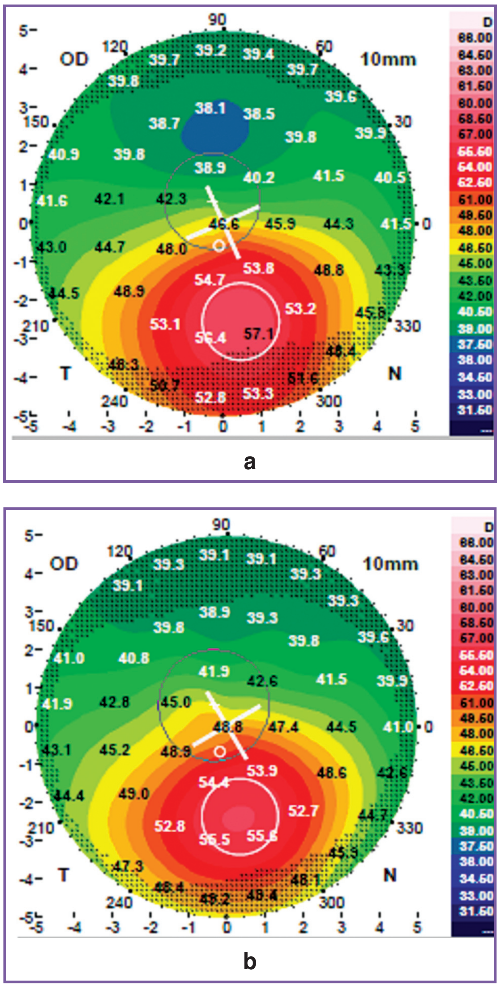

Figure 2. Computerized corneal topography findings of patient X.: (a) before treatment; (b) 6 months after femtosecond crosslinking

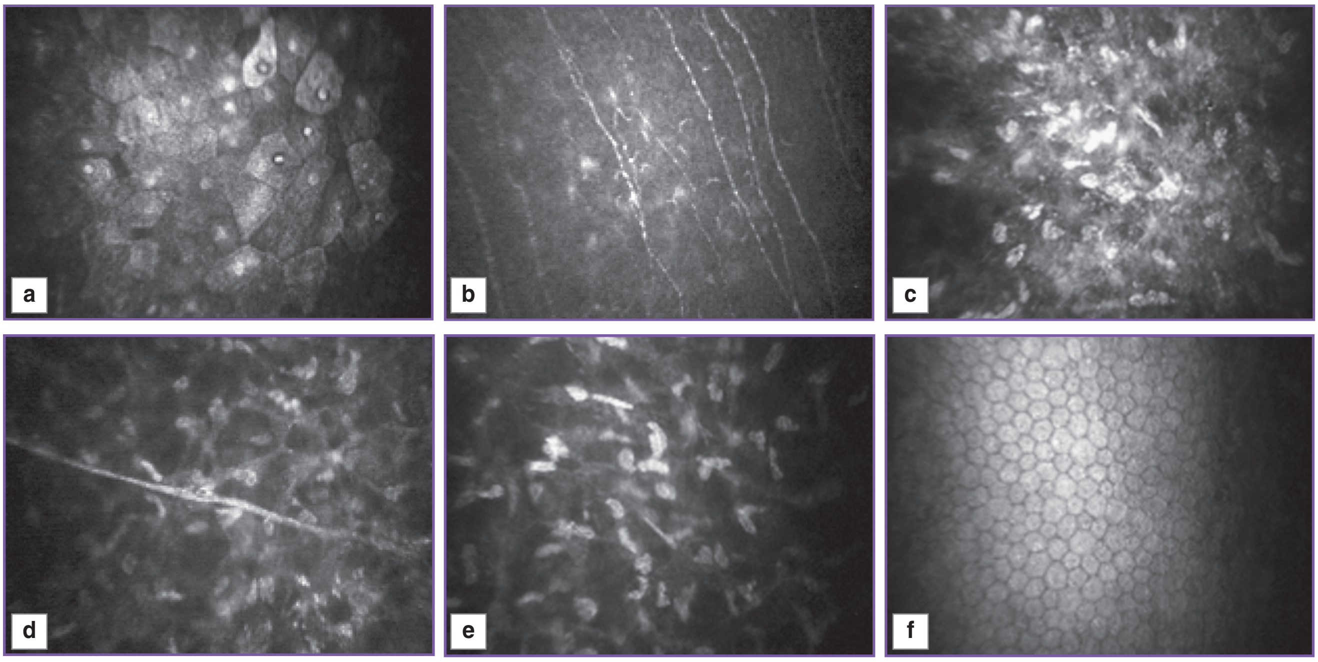

Figure 3. Confocal microscopy of the cornea 6 months after crosslinking: (a) surface epithelium cells; (b) nerve fibers of subepithelial plexus; (c) the superficial stroma with "active" keratocytes; (d) middle stroma; (e) posterior stroma keratocytes; (f) endothelium

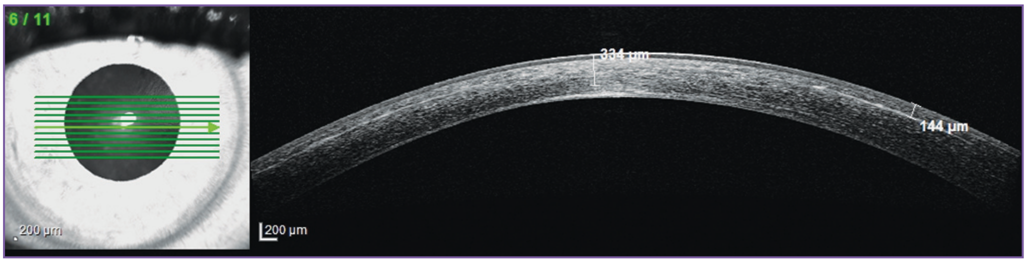

Figure 4. Optical coherence tomography of the cornea 6 months after femtosecond crosslinking; visualized are: 144 µm deep intrastromal pocket and the corneal tissue hyper-reflectivity at the depth up to 334 µm (exposed to cross-linkage effect) with the demarcation line

crosslinking effect due to good absorption of ultraviolet radiation, protection of the corneal endothelium, the crystalline lens and the retina.

Conclusion. Femtosecond corneal collagen crosslinking is a procedure improving functional indexes in keratoconus and making its progression slow down considerablly.

Study Funding. The study was supported by Russian Foundation for Basic Research (grant No.15-29-03874).

Conflicts of Interest. The authors have no conflicts of interest associated with this research.

References

1. Randleman J.B., Dupps W.J. Jr, Santhiago M.R., Rabinowitz Y.S., Koch D.D., Stulting R.D., Klyce S.D. Screening for keratoconus and related ectatic corneal disorders. *Cornea* 2015; 34(8): e20–e22, http://dx.doi.org/10.1097/ico.0000000000000500.

2. Naderan M., Shoar S., Kamaleddin M.A., Rajabi M.T., Naderan M., Khodadadi M. Keratoconus clinical findings according to different classifications. *Cornea* 2015; 34(9): 1005–1011, http://dx.doi.org/10.1097/ico.0000000000000537.

3. Raiskup F., Spoerl E. Corneal crosslinking with riboflavin and ultraviolet A. I. Principles. *Ocul Surf* 2013; 11(2): 65–74, http://dx.doi.org/10.1016/j.jtos.2013.01.002.

4. Wollensak G., Spoerl E., Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol* 2003; 135(5): 620–627, http://dx.doi.org/10.1016/S0002-9394(02)02220-1.

5. Beshtawi I.M., O'Donnell C., Radhakrishnan H. Biomechanical propreties of corneal tissue after ultraviolet-A-riboflavin crosslinking. *J Cataract Refract Surg* 2013; 39(3): 451–462, http://dx.doi.org/10.1016/j.jcrs.2013.01.026.

6. Alió J.L., Toffaha B.T., Piñero D.P., Klonowski P., Javaloy J. Cross-linking in progressive keratoconus using an epithelial debridement or intrastromal pocket technique after previous corneal ring segment implantation. *J Refract Surg* 2011; 27(10): 737–743, http://dx.doi.org/10.3928/1081597x-20110705-01.

7. Wollensak G., Hammer C.M., Spör E., Klenke J., Skerl K., Zhang Y., Sel S. Biomechanical efficacy of collagen crosslinking in porcine cornea using a femtosecond laser pocket. *Cornea* 2014; 33(3): 300–305, http://dx.doi.org/10.1097/ico.0000000000000059.

8. Kılıç A., Kamburoglu G., Akıncı A. Riboflavin injection into the corneal channel for combined collagen crosslinking and intrastromal corneal ring segment implantation. *J Cataract Refract Surg* 2012; 38(5): 878–883, http://dx.doi.org/10.1016/j.jcrs.2011.11.041.

9. Kanellopoulos A.J. *Cornea collagen crosslinking with intralase assisted intracorneal riboflavin administration*. Paper presentation at the subspecialty day of the American Academy of Ophthalmology. New Orleans, LA.; 2007.

10. Sugar A. Ultrafast (femtosecond) laser refractive surgery. *Curr Opin Ophthalmol* 2002; 13(4): 246–249, http://dx.doi.org/10.1097/00055735-200208000-00011.

11. Juhasz T., Loesel F.H., Kurtz R.M., Horvath C., Bille J.F., Mourou G. Corneal refractive surgery with femtosecond lasers. *EEE J Sel Top Quantum Electron* 1999; 5(4): 902–910, http://dx.doi.org/10.1109/2944.796309.

12. Dong Z., Zhou X. Collagen cross-linking with riboflavin in a femtosecond laser-created pocket in rabbit corneas: 6-month results. *Am J Ophthalmol* 2011; 152(1): 22–27, http://dx.doi.org/10.1016/j.ajo.2011.01.010.

13. Pashtaev N.P., Pozdeeva N.A., Zotov V.V., Tihonov N.M. Comparative study of the biomechanical effect of femtocrosslinking in porcine cornea. *Fundamentalnie issledovania* 2015; 1(6): 1217–1221.