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A COMPARATIVE STUDY OF BOWMAN LAYER TRANSPLANTATION RESULTS WITHOUT AND AFTER ULTRAVIOLET CROSSLINKING IN ADVANCED KERATOCONUS

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✧ ***Aim.*** A comparative study of Bowman layer transplantation (BLT) after its ultraviolet (UV) crosslinking and BLT without preliminary UV crosslinking in patients with advanced keratoconus (KC) stages III to IV. ***Materials and methods.*** There were 30 patients aged 14 to 37 years with KC III–IV stages. The first group included 15 patients who underwent BLT without prior UV crosslinking. The second group included 15 patients who underwent BLT after UV crosslinking. The criteria for inclusion of patients in the study were: progressive KC, with corneal thinnest point (CTP) without epithelium of 400 μm or less, a maximum keratometric index (K_{max}) of 58 D and more, with patient satisfied by his visual acuity in a scleral contact lens (SCL) and refusing keratoplasty. ***Results.*** In comparison with preoperative data in both groups, K_{max} decreased by an average 0.6 ± 0.5 D, and CTP increased in the first group by an average of 41.5 ± 16.3 μm, and in the second group by an average of 31,9 ± 9.2 μm. Best corrected visual acuity (BCVA) did not change. ***Conclusion.*** During the follow-up of 26.6 ± 6.2 (from 6 to 36) months, CTP and K_{max} indices remained stable in operated patients, which indicates the arrest of KC progression after BLT with crosslinking and without it. The preservation of endothelial cell density and BCVA values indicates the safety of both methods.

✧ ***Keywords:*** donor cornea; keratoplasty; corneal transplantation; Bowman layer transplantation; keratoconus; crosslinking.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ РЕЗУЛЬТАТОВ ТРАНСПЛАНТАЦИИ БОУМЕНОВОГО СЛОЯ БЕЗ И ПОСЛЕ КРОССЛИНКИНГА ПРИ ПРОГРЕССИРУЮЩЕМ КЕРАТОКОНУСЕ

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✧ ***Цель работы.*** Сравнительное изучение трансплантации боуменового слоя (ТБС) после его кросслинкинга и ТБС без его предварительного кросслинкинга у пациентов с прогрессирующим кератоконусом (КК) III–IV стадии. ***Материалы и методы.*** Под наблюдением находились 30 пациентов в возрасте от 14 до 37 лет с кератоконусом III–IV стадии. В первую группу вошли 15 пациентов, которым была проведена ТБС без его предварительного кросслинкинга. Во вторую группу вошли 15 пациентов, которым была проведена ТБС после его кросслинкинга. Критериями включения пациентов в исследования явились: прогрессирующий КК, с минимальной толщиной роговицы ($ТР_{min}$) без эпителия 400 мкм и менее, максимальным кератометрическим показателем (K_{max}) 58 D и более, с удовлетворяющей пациентов остротой зрения в жёсткой склеральной контактной линзе и отказ пациента от кератопластики.

Результаты. В сравнении с дооперационными данными в обеих группах K_{max} уменьшился в среднем на 0,6 ± 0,5 D, а TP_{min} увеличилась в первой группе в среднем на 41,5 ± 16,3 мкм, во второй группе — в среднем на 31,9 ± 9,2 мкм. Максимально корригируемая острота зрения (МКОЗ) осталась неизменной. ***Заключение.*** В имеющиеся сроки наблюдения 26,6 ± 6,2 мес. у прооперированных пациентов показатели TP_{min}, K_{max} остаются стабильными, что свидетельствует о купировании прогрессирования КК после ТБС с кросслинкингом и без него. Сохранение величин плотности эндотелиальных клеток, МКОЗ свидетельствует о безопасности обеих методик.

✧ ***Ключевые слова:*** роговица донора; кератопластика; трансплантация роговицы; трансплантация боуменового слоя; кератоконус; кросслинкинг.

INTRODUCTION

Keratoconus (KC) is characterized by a progressive thinning of the cornea, the development of irregular astigmatism, and higher order aberrations that occur more often in the second decade of a human's life. These manifestations cause a significant decrease in the visual acuity and quality of life [1, 2]. KC affects all the ethnic populations of both genders; however, men are often more affected than women [3]. There are various theories of the onset and progression of KC, namely genetic [4, 5], endocrine [6, 7], metabolic [8, 9], ecological [10], immunological [11, 12], and allergic [13, 14] conditions, along with systemic diseases [15, 16].

KC affects about 1 of 2,000 people, but its prevalence and incidence can vary in different regions of residence and populations [17–23]. The latest epidemiological studies suggest that there is a global increase in the incidence and the prevalence of KC [18, 20–23].

Refractive error at various stages of KC is most frequently corrected with contact lenses [24, 25]. In case of a poor tolerance of contact correction or dissatisfaction with visual acuity in lenses, the implantation of intrastromal ring segments is performed to remodel the anterior surface of the cornea [26]. Cross-linking is performed to arrest or slow down the disease progression [27]. However, the techniques of cross-linking and intrastromal ring segment implantation are not recommended when the minimum corneal thickness (CP_{min}) is less than 400 μm (without epithelium) and the maximum keratometric index (K_{max}) is more than 58 D [26, 27]. Therefore, cross-linking, according to the Dresden protocol, is contraindicated in the later stages of KC (CP_{min} < 400 μm and K_{max} > 58 D) [27]. At the same time, some authors have demonstrated that the effectiveness of alternative protocols [28–30] is lower than that of the Dresden protocol and is associated with a high risk of complications [31, 32].

One of the specific morphological manifestations of KC is the fragmentation and rupture of Bowman layer (BL). BL in KC has an uneven thickness and is much thinner in the normal corneas than in the affected ones [33, 34]. In 2014, a group of scientists, based upon the specific changes in BL, put forward a hypothesis that the transplantation of BL can stop the progression of KC [35]. In our opinion, the proposed approach was a scientifically substantiated and promising technique; therefore, we tested the technology of BL transplantation (BLT) [36].

Considering the literature data on the effectiveness of BLT and collagen cross-linking, as well as our own BLT results, we considered it natural and appropriate to combine these two complementary techniques. Some factors (BL, like the stroma, consists of collagen; because of anatomical features, the structure of the graft may include anterior layers of the stroma) were the theoretical prerequisites for the effectiveness of BLT with its cross-linking. Considering this aspect, the effectiveness of BLT with its cross-linking should have an even greater stabilizing effect than BLT without cross-linking.

The purpose of this research is to study the comparison between the effectiveness of BLT after its cross-linking and BLT without its preliminary cross-linking in patients with advanced KC (stages III–IV).

MATERIAL AND METHODS

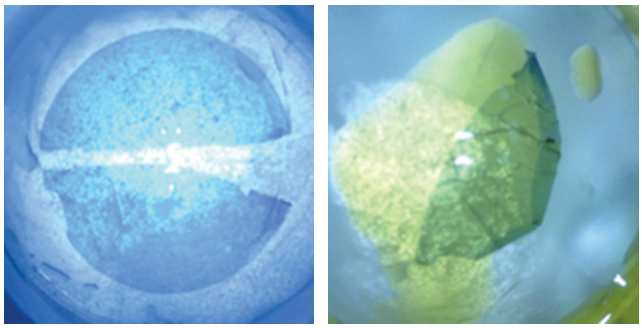
Before clinical studies, to study the level of cross-linking collagens before and after BL cross-linking, we performed differential scanning calorimetry using a Phoenix DSC204 calorimeter (Netzsch, Germany) on the isolated BL samples. We conducted this study at the Department of Bioengineering and Bioinformatics, M.V. Lomonosov Moscow State University.

The detachment of BL from the underlying stroma was performed at the Eye Tissue Bank of the Helmholtz National Medical Research Center of Eye Diseases. A BL graft with a 8 mm diameter was formed from the detached BL with a donor cornea cutter (Barron Vacuum Punch from the donor; Kat-

ena Products Inc, USA) and preserved in the Borzenok – Moroz medium. After 24 hours, the BL sample was divided into two equal parts using a corneal cutter for the partial transplantation of Descemet's membrane with endothelium [37]. One half of the sample was immersed in a solution of normotonic riboflavin (riboflavin 0.1% and dextran 20%) for 15 min and then cross-linked for 15 min (UV-X, 1000, IROC AG, Switzerland) at a wavelength of 370 μm, a power flux density of 3.0 mW/cm^2, and a radiant exposure of 2.7 J/cm^2. The other half of the sample was left intact (Fig. 1).

During day 1, both halves of the sample were stored in the Borzenok – Moroz medium, after it all the samples were subjected to differential scanning calorimetry. The analysis showed that the denaturation temperature of the samples of the experimental BL was higher (68.8 °C ± 0.15 °C) than that of the control BL, which had an average value of 67.1 °C ± 0.2 °C. The detected difference in temperatures at the start and end of the denaturation process between the groups of experimental and control BL samples indicated an increase in the level of cross-links after cross-linking in the group of experimental samples (Fig. 2).

Hence, we can declare that cross-linking has occurred in the isolated BL samples. The obtained results enabled the initiation of the clinical testing of the technique.



a *b*

Fig. 1. Isolated Bowman layer: *a* – intact samples, *b* – experimental sample after crosslinking

Рис. 1. Изолированный боуменовый слой: *a* — интактные образцы, *b* — опытный образец после кросслинкинга

Since 2016, 30 patients, including 27 men and 3 women aged 14–37 years with KC stages III–IV, have been under our supervision. The patients were distributed into two equal groups. Group 1 included 15 patients (15 eyes) who underwent BLT according to the previously described technique [36]. In group 1, the average age of patients was 24.9 ± 7.2 years, the average preoperative CP_{min} was 338.4 ± 73 μm, the average K_{max} was 66.4 ± 4.1 D, and the average best-corrected visual acuity (BCVA) was 0.69 ± 0.18.

Group 2 included 15 patients (15 eyes) who underwent BLT after its cross-linking. The average age

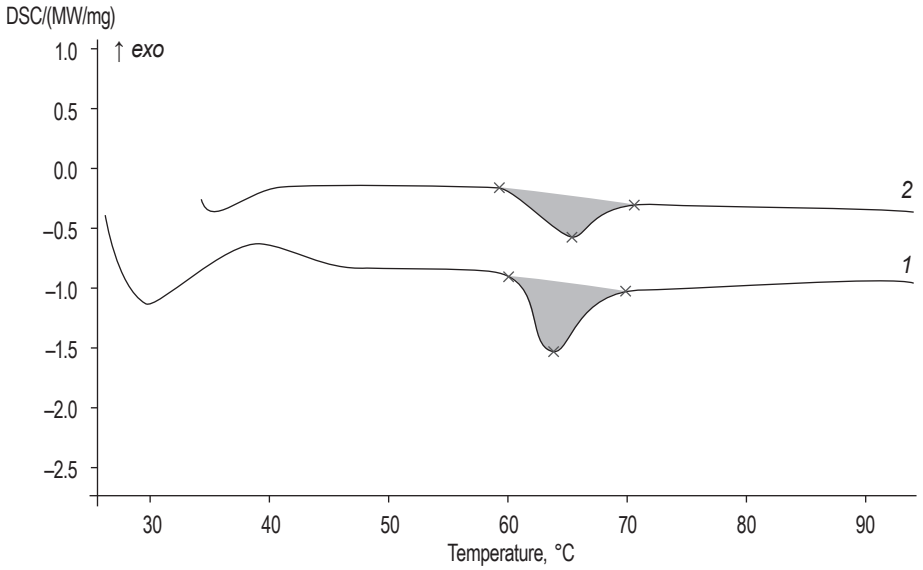


Fig. 2. Differential scanning calorimetry of Bowman layer control (*1*) and experimental (*2*) samples

Рис. 2. Дифференциальная сканирующая калориметрия образцов контрольного (*1*) и опытного (*2*) БС. *1* — Комплексный пик: площадь — –14,78 Дж/г; пик – 63,7 °C; начало 61,5 °C; конец — 67,2 °C; ширина 4,8 °C (37000 %); высота — 0,5656 мВт/мг. *2* — Комплексный пик: площадь — –8,289 Дж/г; пик — 65,4 °C; начало 61,5 °C; конец — 68,1 °C; ширина 5,2 °C (37000 %); высота — 0,3034 мВт/мг

of the patients was 28.7 ± 4.6 years, the CP_{min} of the cornea was 353 ± 39.8 μm, the average K_{max} was 65 ± 3.4 D, and the average BCVA was 0.73 ± 0.15. The criteria for the inclusion of patients in the studies were advanced KC, CP_{min} without an epithelium of 400 μm or less, K_{max} of 58 D and more, corrected visual acuity using a rigid scleral contact lens (RSCL) that was satisfactory for the patients, and refusal of keratoplasty by the patient. In our study, contact lens tolerance and satisfaction with vision were registered in all the patients. For this reason, all the patients refused corneal transplantation.

Comorbidities included atopic dermatitis with multiple scratching and hemorrhagic crusting, blepharitis, allergic conjunctivitis, and Down syndrome. The vast majority of patients had a habit of uncontrollably rubbing their eyes and sleeping with compression on the sore eye. In all the cases, we had keratopachymetric indices of the examined eyes from 6 to 12 months before the study. We examined the patients before surgery, 5 days, 1, 3, 6, 12 months post-surgery, and then annually. The average follow-up period was 26.6 ± 6.2 months (6–36 months). Tables 1 and 2 present the demographic and preoperative keratopachymetric indices of groups 1 and 2, respectively.

The study was conducted in compliance with the provisions of Helsinki Declaration after obtaining the informed consent from the patients and the approval of the Ethical Committee of the Helmholtz National Medical Research Center of Eye Diseases of November 17, 2016 (extract from Protocol No. 33/3). The patients underwent standard ophthalmological examinations at the term according to the study protocol. Mandatory studies included visometry under mesopic conditions without correction, with correction using glasses and RSCL (before surgery and not earlier than three months after surgery), keratoanalysis (Galilei G6, Ziemer Ophthalmic Systems AG, Switzerland), and optical coherence tomography of the cornea (Spectralis, Heidelberg, Germany). The endothelial cells were counted in a manual mode using a fixed frame technique after obtaining the images by the contact method on a ConfoScan 4 device (Nidek Co. Ltd., Japan). Photographs and video recordings were taken at each visit of the patients. To analyze the results in both groups, K_{max} and pachymetric images were assessed along with the obligatory identification of the localization and CP_{min} value of the cornea (Tables 1 and 2).

All surgeries were performed by a single surgeon under local instillation and subconjunctival anesthesia.

Table 1 / Таблица 1

Demographic and preoperative keratopachymetric indices of group 1
Демографические и дооперационные кератопахиметрические показатели группы 1

Patient's No./gender/age, years	Follow-up period, mon.	CT_{min}, μm	K_{max}, D	ECD, mm^2
1/M/25	36	405	64.6	2552
2/M/30	29	409	67.6	2791
3/M/14	27	367	69.1	2487
4/M/38	25	335	70.2	2910
5/M/23	24	185	69.1	2762
6/F/17	23	415	67	3012
7/F/37	20	265	53.4	2764
8/M/17	19	367	64.9	2291
9/M/17	18	403	65.6	2670
10/M/29	18	409	68.1	2791
11/M/19	15	397	66.3	2940
12/M/29	15	312	64.2	2487
13/M/25	12	258	69.1	2347
14/M/26	10	254	66.5	2762
15/M/27	6	295	70.5	2856
Average 24.9 ± 7.2	19.8 ± 7.8	338.4 ± 73	66.4 ± 4.1	2694 ± 216

Note. CT_{min} – minimum corneal thickness, K_{max} – maximum keratometric index, ECD – endothelial cell density.

Table 2 / Таблица 2

Demographic and preoperative keratopachymetric indices of group 2
Демографические и дооперационные кератопахиметрические показатели группы 2

Patient's No./gender/age, years	Follow-up period, mon.	CT_{min}, μm	K_{max}, D	ECD, mm^2
1/M/29	33	388	63.7	2645
2/F/35	32	356	59.5	2489
3/M/27	31	265	70.1	2310
4/M/26	31	367	64.7	2528
5/M/25	29	423	63.7	2673
6/M/21	29	397	62.6	3100
7/M/22	29	375	59.1	2653
8/M/32	27	324	65.7	2871
9/M/25	21	376	66	2991
10/M/38	15	359	61.2	2653
11/M/27	12	298	66.2	2871
12/M/32	10	324	68.6	2879
13/M/26	10	367	69.3	2843
14/M/29	9	342	67.2	3019
15/M/30	6	343	68.5	2461
Average 28.7 ± 4.6	21.6 ± 10	353 ± 39.8	65 ± 3.4	2732 ± 228

Note. CT_{min} – minimum corneal thickness, K_{max} – maximum keratometric index, ECD – endothelial cell density.

In all cases, donor corneoscleral buttons without Descemet's membrane were the source of the graft. The BL was prepared on the evening of the surgery in the Eye Tissue Bank of the Helmholtz National Medical Research Center of Eye Diseases. The prepared BL was stored in the Borzenok – Moroz medium at a temperature of +4 °C.

SURGERY TECHNIQUE

The corneoscleral button was fixed on an artificial anterior chamber (Katena, USA). After mechanical deepithelialization, the cornea was marked using a circular marker of 9 mm and trypan blue. A notch was made 1–2 mm peripheral from the cornea, using a keratotomy diamond knife with a micrometer (RF patent No. 2647197). Thereafter, BL was delaminated from the underlying stroma with flat microsurgical forceps. The graft was immersed for 30 s in a 70% ethanol solution for complete deepithelialization. Then, it was immersed for 30 s in a balanced saline solution (BSS) (Fig. 3) and, finally, in a preservative until the surgery.

On the day of surgery, for group 2, BL was cross-linked with a UV-X 1000 apparatus (IROC AG, Switzerland). Thereafter, BL was placed in a solution of normotonic riboflavin (riboflavin 0.1% and dextran 20%) for 15 min. Next, it was straightened on a contact lens and irradiated at a wavelength of 370 μm, a power flux density of 3.0 mW/cm^2, and the radiant exposure of 2.7 J/cm^2 for 15 min while ensuring the instillation of riboflavin with 1 drop in every 2 min (RF patent No. 2645931).

The exposure and irradiation times were chosen empirically and determined by the minimum graft thickness. Because of this process, significantly less time is required for the infiltration of riboflavin and the absorption of the necessary dose of ultraviolet radiation. The absence of endothelial cells and limbal stem cells in the BL graft (in contrast to the patient's full thickness cornea) makes the choice of cross-linking parameters more distinguishable. Then,

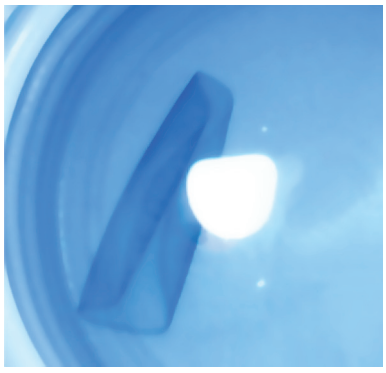


Fig. 3. Isolated Bowman layer in BSS solution

Рис. 3. Изолированный боуменовый слой в сбалансированном физиологическом растворе BSS

the graft was stained with 0.06% trypan blue solution (Vision BlueTM, DORC International). Next, it was washed in BSS solution and placed on a contact lens. With a corneal cutter (Barron Vacuum Punch, Katena, USA), the graft with a diameter of 8–9 mm was excised.

The conjunctiva was incised along the limbus in the patient's eye at the meridian of 11–13 hours. After cauterization of the scleral vessels, an incision of 3 mm was made with a blade up to half of the scleral depth 1–2 mm from the limbus and parallel to it. Corneal paracentesis of 0.9 mm was performed with a lancet knife, by which the anterior chamber was filled with air. An intrastromal pocket was formed within the limbal ring with a splitter knife, starting from the 12-hour meridian. Thereafter, the air was partially removed from the anterior chamber. The folded BL graft was inserted into the formed pocket of the recipient's cornea and, by manipulations *ab interno*, was straightened with the epithelial side up and centered in the corneal layers. The surgery was finished with the application of one conjunctival suture.

RESULTS

No intraoperative complications were registered in any of the cases. A standard course was followed in the surgeries of all the cases. The greatest difficulty was the recipient's thin cornea exfoliation at the KC apex. The postoperative period in all the cases was uneventful. In group 2, one patient had a corneal haze in the optical zone after surgery (Fig. 4).

Tables 3 and 4 present the postoperative keratopachymetric indices of groups 1 and 2, respectively. The comparison of preoperative data in both groups demonstrated that K_{max} decreased on average by 0.6 ± 0.5 D, and CP_{min} increased by an average of 41.5 ± 16.3 μm in group 1 and by an average of 31.9 ± 9.2 μm in group 2. The absence of negative dynamics in the keratopachymetric values of both the groups after BLT indicates a stabilizing effect of the BLT technique on the progression of KC, both with and without cross-linking. Visual acuity without correction improved after BLT in two patients from 0.03 ± 0.02 to 0.08 ± 0.01 in group 1 and from 0.05 ± 0.02 to 0.07 ± 0.02 in group 2. At the same time, the BCVA in scleral lenses had

Table 3 / Таблица 3

Postoperative keratopachymetric indices of group 1
Послеоперационные кератопахиметрические показатели в группе 1

Patient's number	Follow-up period (mon.)	CT_{min}, μm				K_{max}, D			
		1 mon.	6 mon.	Last examination	Difference between preoperative and recent research	1 mon.	6 mon.	Last examination	Difference between preoperative and recent research
1	36	452	441	438	36	64.4	65.2	64.1	–0.5
2	29	432	426	431	22	67.3	65.5	66.2	–1.4
3	27	420	389	387	20	69	68.7	68	–1.1
4	25	390	384	377	42	70.7	70.3	69.8	–0.4
5	24	289	276	255	70	69.5	69	68.7	–0.4
6	23	492	449	451	36	68.1	67.2	67.2	–0.9
7	20	357	312	291	26	54	53.3	53.5	+0.1
8	19	406	394	400	33	62.9	63.1	64.1	–0.8
9	18	447	449	452	49	66.6	65.5	64.9	–0.7
10	18	470	467	461	52	66.7	66.8	67.9	–0.2
11	15	423	425	431	34	66.3	66.2	66	–0.3
12	15	373	365	373	61	63.7	64.6	64.1	–0.1
13	12	272	270	281	23	69	68.8	68.6	–0.5
14	10	310	289	321	67	65.5	65.7	65.5	–1
15	6	352	347	347	52	69.9	69.8	68.9	–1.6
Average	27.7 ± 8.9	392 ± 66.1	378 ± 66.7	379 ± 67.4	41.5 ± 16.3	66.2 ± 4.1	65.4 ± 3.6	65.8 ± 3.9	–0.6 ± 0.5

Note. CT_{min} – minimum corneal thickness, K_{max} – maximum keratometric index

not changed in any case. The endothelial cell density (ECD) remained unaltered in both groups, as compared with the preoperative data. The ECDs were 2,678 ± 209 mm^2 and 2,810 ± 213 mm^2 in groups 1 and 2, respectively. The obtained data indicate the functional safety of BLT. In all cases, the graft was evenly located in the corneal pocket, without folds, at an equal distance from the posterior surface of the cornea.

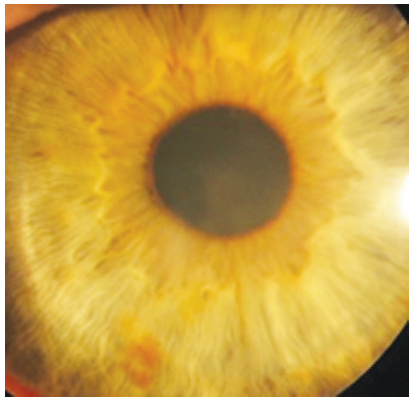


Fig. 4. Postoperative corneal haze in a group 2 patient

Рис. 4. Послеоперационный хейз роговицы у пациента группы 2

DISCUSSION

Throughout the twentieth century, penetrating keratoplasty (PKP) was the gold standard for the treatment of advanced KC [38]. In the early twenty-first century, there was a breakthrough in keratotransplantation because of selective keratoplasty and cross-linking [27, 39]. Due to the dynamic development of the cross-linking technique, a significant decrease in the frequency of keratoplasty in KC patients can be expected, the effectiveness of which was confirmed by randomized controlled trials [40–42]. Deep anterior lamellar keratoplasty (DALK) in patients with KC significantly increases the biological efficiency of keratoplasty because of the exclusion of endothelial rejection reaction. However, the problems associated with unpredictable visual acuity, sutures, and stromal rejection reactions are similar to those of PKP [43].

The development of modern RSCLs providing high BCVA at advanced stages of KC, when cross-linking

Table 4 / Таблица 4

Postoperative keratopachymetric indices of group 2
Послеоперационные кератопахиметрические показатели в группе 2

Patient's number	Follow-up period (mon.)	CT_{min}, μm				K_{max}, D			
		1 mon.	6 mon.	Last examination	Difference between preoperative and recent research	1 mon.	6 mon.	Last examination	Difference between preoperative and recent research
1	33	449	417	412	24	64.1	64.4	63.3	–0.4
2	32	430	399	402	46	60.2	60.8	59.2	–0.3
3	31	376	291	301	36	69.9	69.8	68.6	–1.5
4	31	399	387	385	18	65.2	65.9	64.5	–0.2
5	29	478	451	449	26	63.9	63.5	63.1	–0.6
6	29	465	426	429	32	61.6	62.7	61.5	–1.1
7	29	410	391	395	20	58.4	58.8	58	–1.1
8	27	372	361	366	42	65.6	65.6	65.6	–0.1
9	21	461	413	419	43	66.2	65.8	66.2	+0.2
10	15	398	379	381	22	61	60.6	60.8	–0.4
11	12	356	342	339	41	65.6	65.5	66	–0.2
12	10	380	376	366	42	68.2	67.8	67.7	–0.9
13	10	400	391	395	28	69	68.6	68.7	–0.6
14	9	392	382	373	31	66.8	66.1	65.9	–1.3
15	6	388	371	371	28	68.2	68	68.1	–0.4
Average	21.6 ± 10	402 ± 37.6	385 ± 37.5	385 ± 36.5	31.9 ± 9.2	64.9 ± 3.4	64.9 ± 3.1	64.5 ± 3.4	–0.6 ± 0.5

Note. CT_{min} – minimum corneal thickness, K_{max} – maximum keratometric index.

is impossible, as well as probable complications of keratoplasty, contributed to the emergence of the BLT technique. This technique is an alternative to PKP and DALK in patients with advanced KC and has an aim of maximum or lifelong delay of keratoplasty. The surgery is not aimed at the improvement of visual acuity; however, when the cornea is flattened, visual acuity may improve with this surgery [44].

The undoubted advantage of BLT is its extraocular nature, and, consequently, the exclusion of expulsive bleeding, endophthalmitis, secondary glaucoma, and other complications in the case of intraocular surgery [45]. Acellularity of BL eliminates the rejection reaction, this reduces the period of instillation of glucocorticosteroids to three months, and the microinvasive nature of this technique enables the reduction of the rehabilitation period to two weeks. Surgical treatment is possible under local anesthesia, thereby expanding the indications for patients who have systemic comorbidities.

The need to use BL only enables the transplantation of the Descemet's membrane with endothelium in patients with endothelial dystrophy, thereby applying the concept of rational use of donor material.

In some cases, cross-linking becomes possible because of the thickening of cornea, without the fear for the integrity of the corneal endothelium. In the case of patient's dissatisfaction with the quality of life or the absence of termination of the progression of KC, there is still a possibility of subsequent PKP/DALK.

Unlike DALK, BLT is a technically simpler and predictable surgery that does not require long training and extensive experience.

CONCLUSION

BLT is a new method of treating patients with advanced KC, which helps in stopping the ectatic process. Our study demonstrated that in the available follow-up period of 26.6 ± 6.2 months (6–36 months) in patients we operated, the CP_{min} and K_{max} indicators remain stable, which indicates the arrest of the progression of KC after BLT with and without cross-linking. The preservation of ECD and BCVA values, as well as the absence of postoperative complications, indicates the safety of both methods. For the first time, we experimentally proved the fact of cross-linking in an isolated BL. Thus, in the long term, BLT after its cross-linking should have an even greater stabilizing effect. However, in the available follow-up period, there are no data to conclude that BLT after cross-linking is a more effective treatment method. Despite this observation, the feasibility of using both the techniques is the optimal and effective way to arrest the advanced KC in the patients with satisfactory visual acuity in RSCL.

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Contribution of authors. O.G. Oganesyan was the author of the idea, conducted clinical examination, surgical treatment and postoperative management of the patients, performed statistical data processing, and wrote the text; V.R. Getadaryan was the author of the idea, conducted clinical examination and postoperative management of the patients, performed statistical data processing, and wrote the text; P.V. Makarov was the author of the idea, was involved in clinical examination and postoperative management of the patients, performed statistical data processing, and wrote the text; P.M. Ashikova was the author of the idea, conducted clinical examination and postoperative management of the patients, as well as statistical data processing, and wrote the text; N.Yu. Ignatieva performed consultation, experimental part of the work, interpreted the results, and wrote the text.

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