SI "THE FILATOV INSTITUTE
OF EYE DISEASES AND TISSUE THERAPY
OF NAMS OF UKRAINE"

Academician V.P.Filatov

Past, Present and Future of Artificial Corneas: Ukraine experience

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[3] Linkoping University, Linkoping, Sweden

Background

- **Around 10 million people worldwide are blind because of corneal diseases[1]**
- **Worldwide shortage of human donor corneas for transplantation**
  - In developed countries like the UK, the estimated need for corneas is approximately 5000, with a shortfall of 1500 (30% are untransplanted)[2]
  - In developing countries, where about 200,000 donor corneas per year are needed but only 30-40,000 are collected[3]
  - Annual need of corneal transplantations in Ukraine – 3000, mean number of human donor cornea transplantations per year - 500
- **High risk of donor cornea rejection in a number of pathologies**
  - Chemical and thermal eye injuries
  - Auto-immune diseases (Stevens-Johnson syndrome, OCP)
  - Previously failed corneal graft

[1] Witcher J, Srinivasan M, Upadhyay MP. Corneal blindness: A global perspective. *Bull World Health Organ.* 2001; 79: 214-21

[2] Gaum L, Reynolds I, Jones MNA, Clarkson AJ, Gillan HL, Kaye SB. Tissue and corneal donation and transplantation in the UK. *Br J Anaesth.* 2012; 108 (suppl 1): i43-i47

[3] Sangwan VS, Gopinathan U, Garg P, Rao GN. Eye Banking in India: A Road Ahead. *JIMSA.* 2010; 23(3): 197-200

History

- Ukrainian ophthalmologist Vladimir Filatov performed one of the first corneal transplantations in human (1912)
- first suggested the use of cadaver corneas for transplantation and elaborated first preservation method - “moist chamber” (1929) *
- Founded Research Institute of Eye Diseases in Odessa (1936). It has continued in the tradition to seek out technologies for treating complex transplantation cases
- **>50000** corneal transplantations were performed at the Institute after WWII





* Filatov VP. Transplantation of the cornea from preserved cadavers' eyes. Lancet 1935;232:1395.

I - KERATOPROSTHESIS

Keratoprosthesis in Filatov Institute

- has been developing, studying and applying since1966 (Puchkovskaya N.A., Iakymenko S.A., Golubenko E.A.)*:
  - original keratoprosthesis construction – “universal separable K-pro” (optical cylinder – PMMA, support – tantalum)
  - original keratoprosthesis implantation method
  - leukoma and keratoprosthesis strengthening methods
  - study of keratoprosthesis complications, development of methods of their prophylaxis and treatment


Puchkovskaya N.A.


Iakymenko S.A.



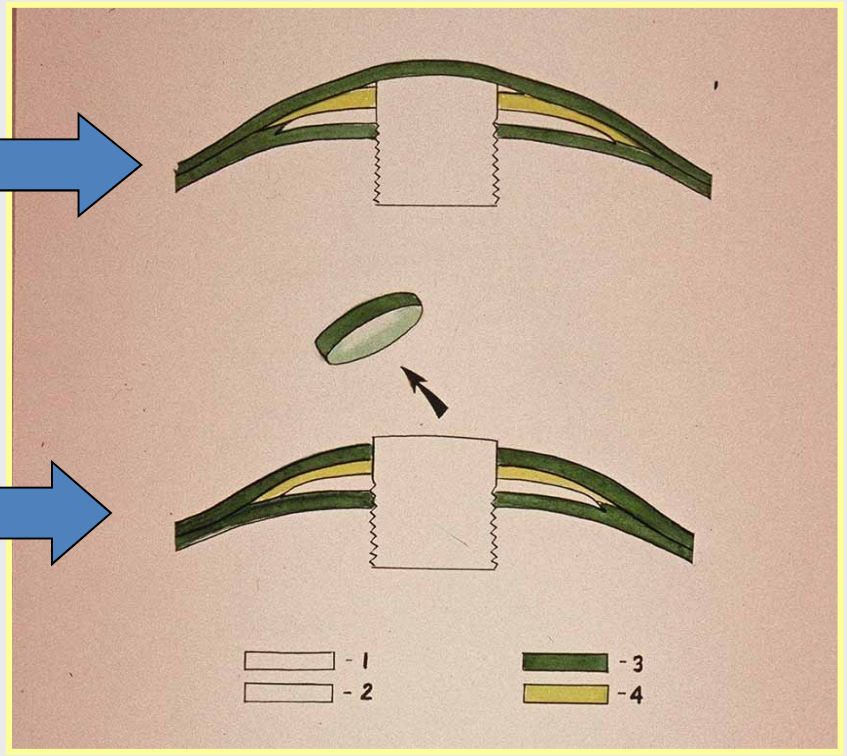
* Iakymenko S. Forty-five years of keratoprosthesis study and application at the Filatov Institute: a retrospective analysis of 1 060 cases *Int J Ophthalmol* 2013;6:375-80.

“Two-stage” method of keratoprosthesis *

1st stage – after division of cornea into anterior and posterior layers only its posterior layers are trephined, where keratoprosthesis is placed. Then keratoprosthesis is covered by non-trephined anterior layers of cornea

2nd stage (in 3-5 months) – only corneal anterior layers over optical cylinder of keratoprosthesis are trephined

Such method allows widely applying intracorneal strengthening of leukomas with different tissues in the same time with keratoprosthesis implantation



1,2 – keratoprosthesis
3 – cornea
4 – intralamellar grafts

* Puchkovskaya NA, Yakimenko SA. Two-stage method of keratoprosthesis. *Oftalmol Zh.* 1978;33:498–500







- before the operation

- after the first stage of keratoprosthesis

(keratoprosthesis in corneal layers)

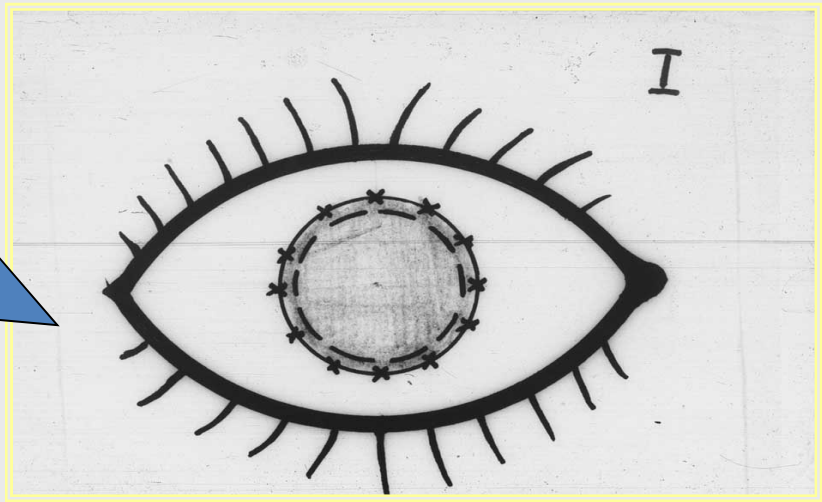
- after the second stage of keratoprosthesis - in 5 months after the first stage (cornea over optical cylinder of keratoprosthesis is trephined)

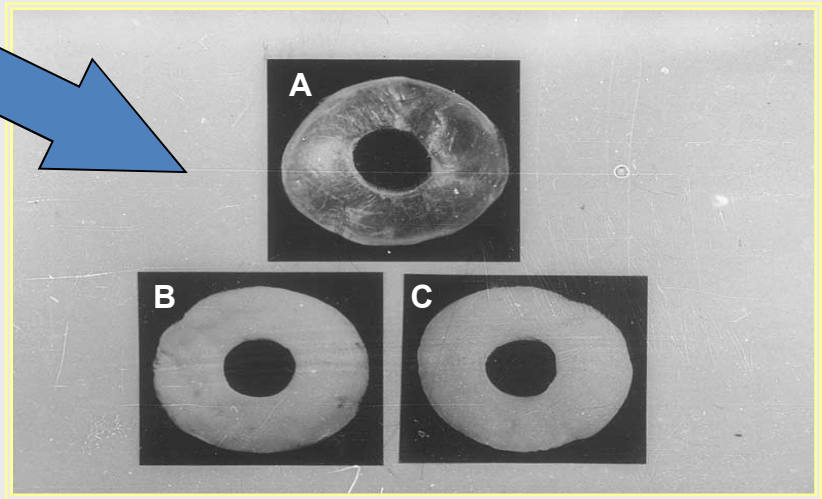
Leukoma strengthening methods *

- **epicorneal**
  - donor cornea
  - patient's oral mucosa

  (is performed 5-6 months before keratoprosthesis)
- **intracorneal**
  - donor cornea (A)
  - patient's auricular cartilage (B)
  - dura mater (C)

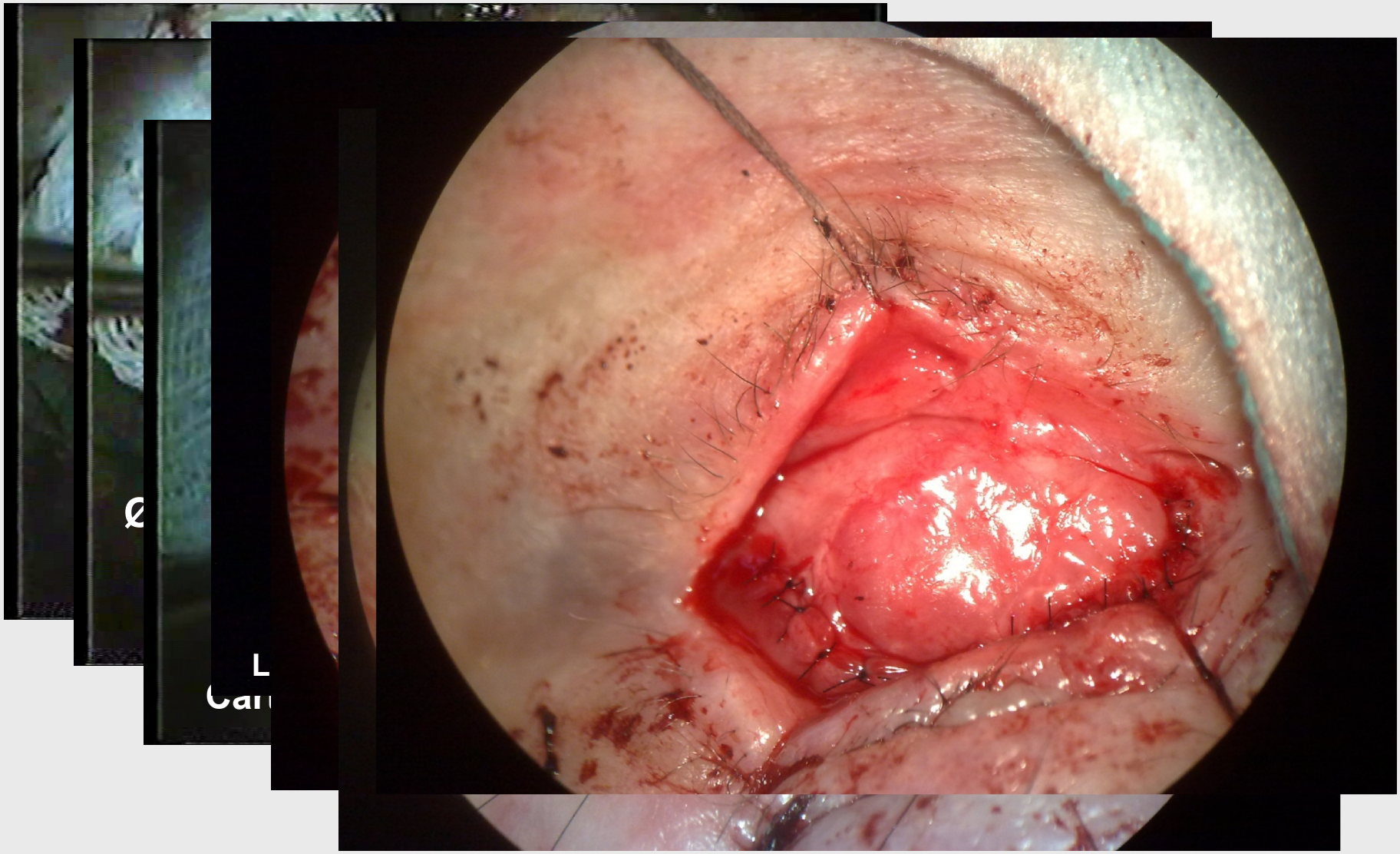
  (is performed during keratoprosthesis implantation)
- **different combinations of these methods**





* Yakimenko SA. Methods for strengthening leukoma during keratoprosthesis and research of their effectiveness in follow-up. *Oftalmol Zh.* 1984; 39:406–410.

Keratoprosthesis implantation



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K-Pro Results

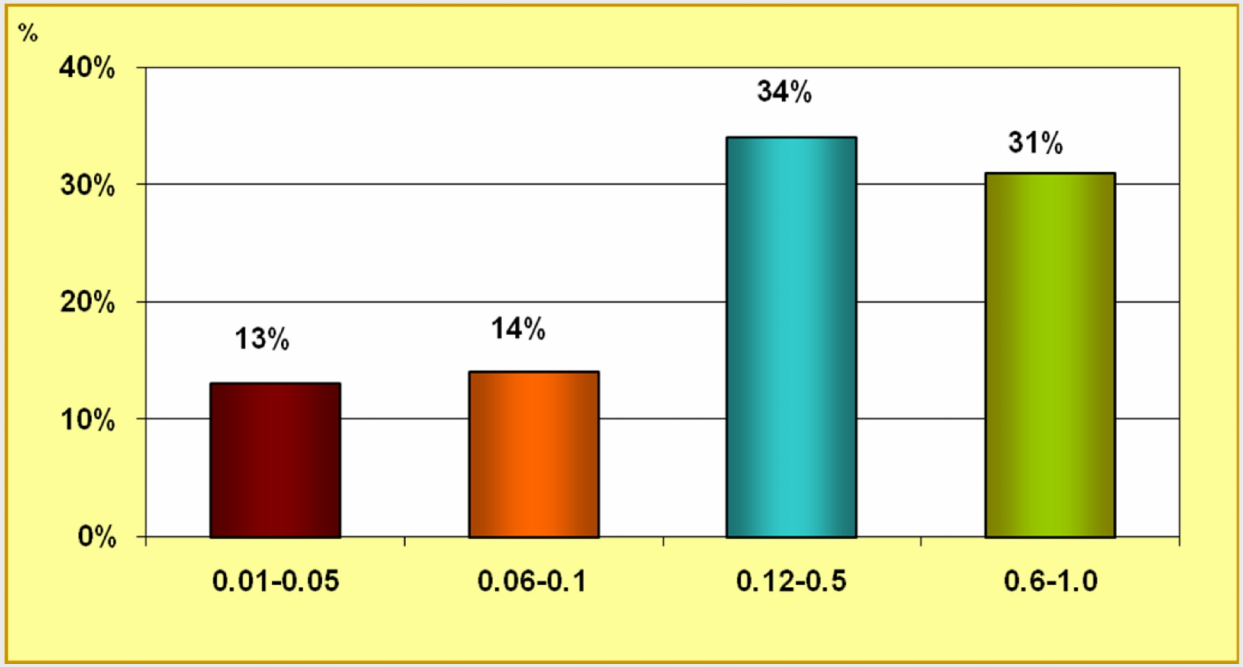
Keratoprosthesis in our Institute was performed on 1060 eyes of 1040 patients with high-risk leukomas of different etiology

Etiology	Number of eyes (%)
Eye burns	725 eyes (68,4%)
Trauma	120 eyes (11,3%)
Keratitis (corneal ulcers), ocular pemphigoid, Stevens-Johnson syndrome	108 eyes (10,2%)
Edematous (aphakic, bullous) keratopathy	107 eyes (10,0%)

K-Pro Results

- Before keratoprosthesis 92% of eyes (962 eyes) had light perception, and 8% (98 eyes) had minimal vision (0.005-0.02)
- Both eyes were blind in 90% of patients (932 patients)
- Period of blindness varied from 1 to 52 years
- Age of patients varied from 11 to 82 years
- Keratoprosthesis was the only possible method for vision restoration in these patients

K-Pro Results



Visual acuity after keratoprosthesis

- Different degree of vision was obtained in 96.5% of eyes (1023 of 1060 eyes)
- Visual acuity after keratoprosthesis more than 0.1 - in 68% of eyes (721 eye)

K-Pro Complications

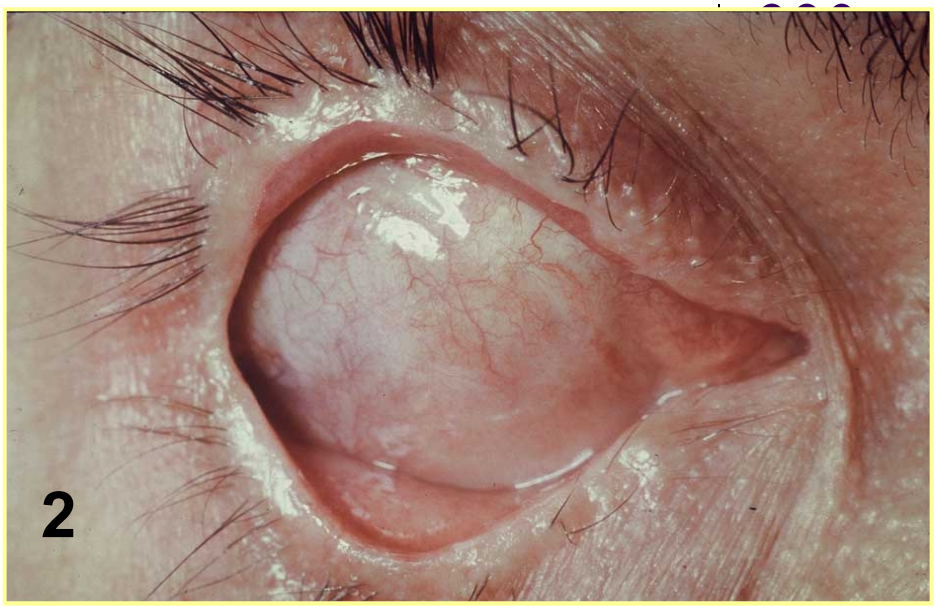
Type of Complication	Years of operation, number of operated eyes			
	1972-1974 70 eyes	1975-1977 157 eyes	1978-1994 543 eyes	1995-2012 290 eyes
Aseptic necrosis of cornea	28.6%	20.4%	8.3%	2.9%
Extrusion of keratoprosthesis	17.1%	9.5%	3.5%	1.5%
Phthisis bulbi (uveitis, aqueous humor filtration)	7.1%	5.7%	2.4%	-
Endophthalmitis	1.4%	5.7%	1.5%	0.5%
Retinal detachment	1.4%	2.5%	1.1%	0.5%
Retrokeratoprosthesis membrane	45.7%	14.6%	13.6%	16.5%
Overgrowth of mucosa over keratoprosthesis	20.0%	19.7%	24.9%	18.3%
Secondary glaucoma	5.7%	1.3%	1.3%	1.5%

K-Pro Results at last follow-up

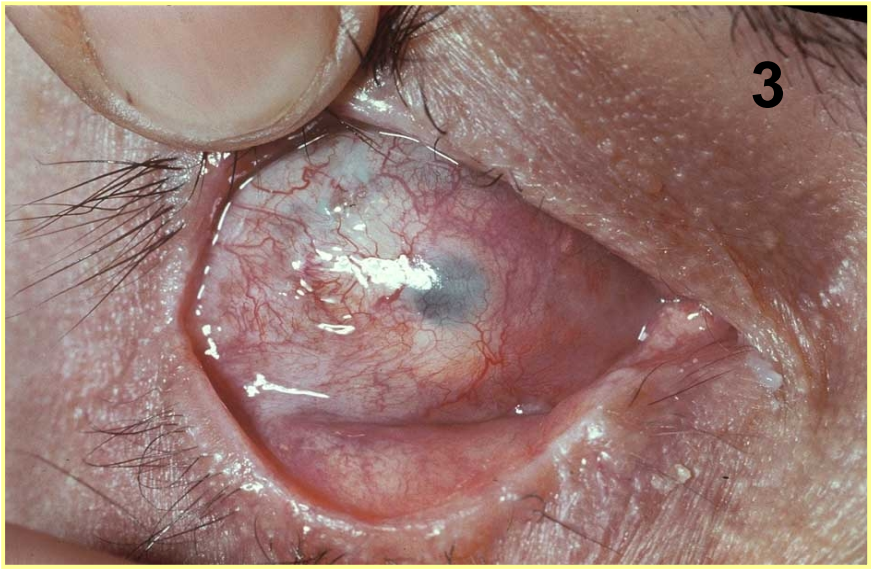
- Vision (0.01-1.0) preserved in 89.5% of operated eyes (953/1060 eyes)
- Good keratoprostheses fixation in cornea was obtained in 93% of operated eyes
- Follow-up was from 1 to 40 years



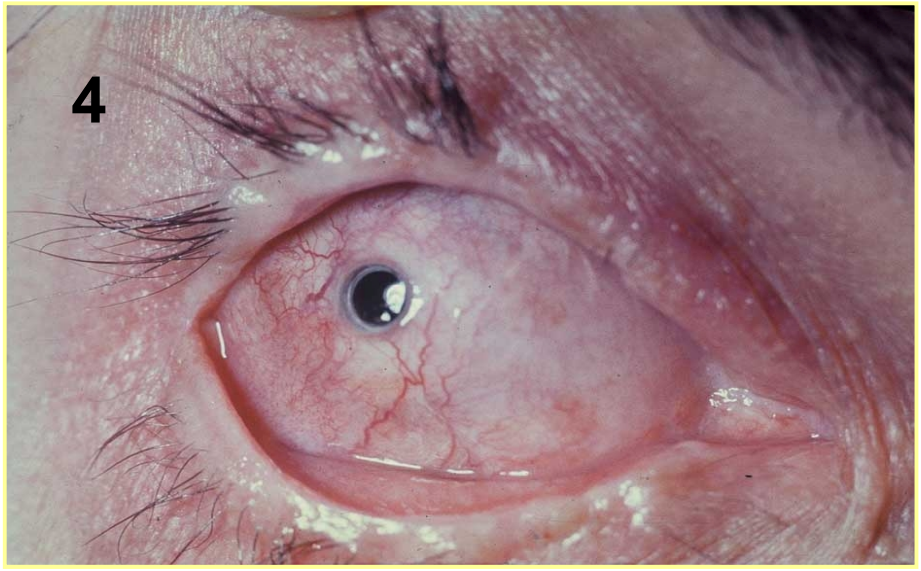
1- preoperative status – both eyes (1975)



2 – RE - after symblepharon repair with oral mucosa plasty (1976)



3 – after the 1st stage of keratoprosthesis (1976)



4 – after the 2nd stage of keratoprosthesis visual acuity = 1.0. Follow up period 34 years

K-Pro
Conclusion

- Keratoprosthesis with use of our technologies is an effective method of vision restoration in patients with leukomas unsuitable for optical corneal transplantation

II - CORNEA XENOTRANSPLANTATION

History

- First studies to adjust animal corneas for transplantation were initiated at Filatov Institute after WW II – pigs, dogs, cats, rabbits, etc. (Filatov V.P., Voino-Yasenetskiy V.V. et al., 1946-1950)

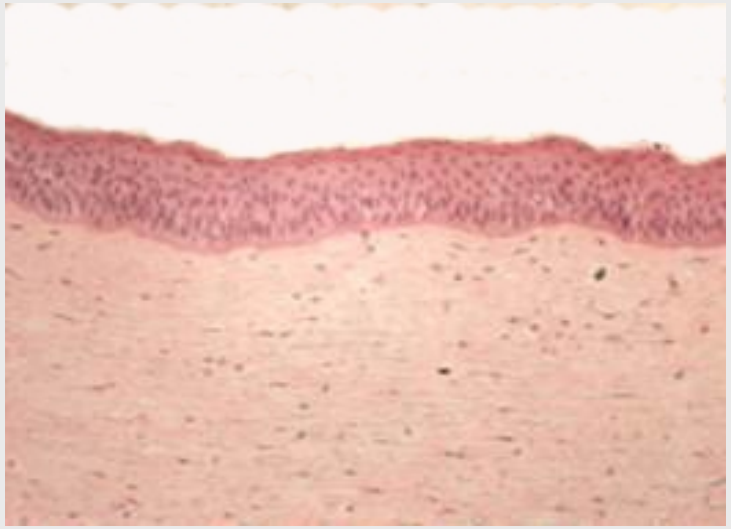
- Vast majority of xenocornea transplantation in animals failed due to extensive graft-versus-host disease

- In collaboration with Ternopil State Medical University project was started to study porcine cornea as possible human donor cornea substitute

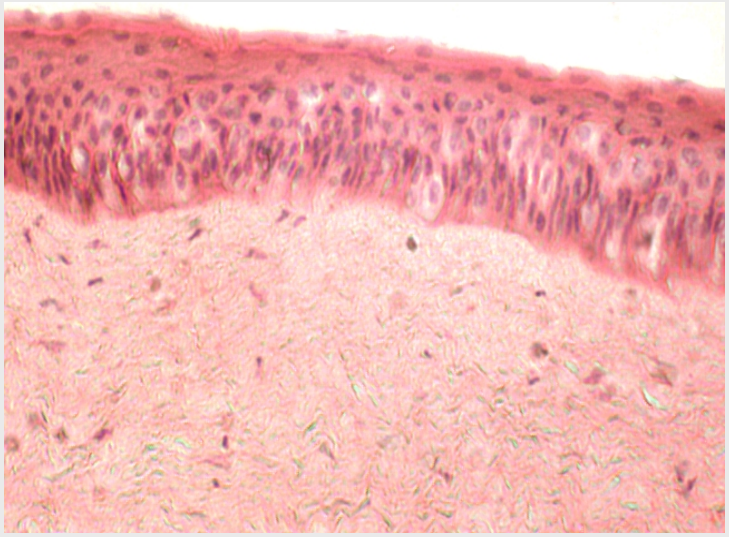


Experiments in vitro

- Influence of different preservation methods on porcine cornea structure and antigen properties was studied
- Cryopreservation at -180ºC with cryoprotector and subsequent lyophilization preserves porcine cornea structure and decreases antigen properties (Gal antigens)



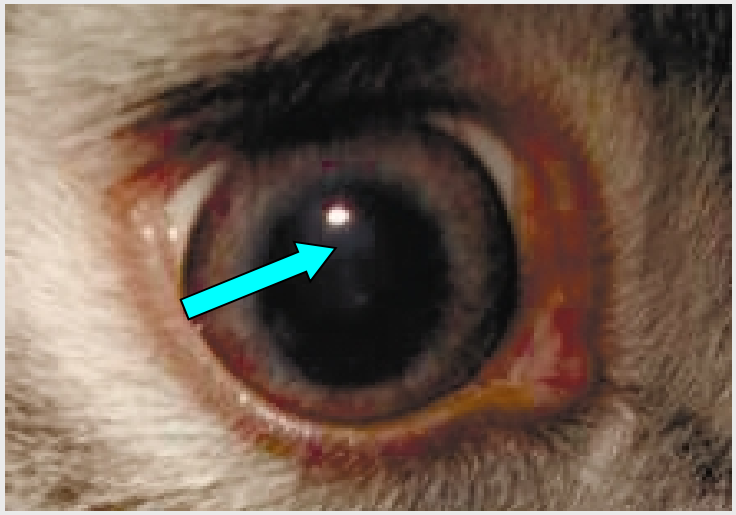
Native porcine cornea

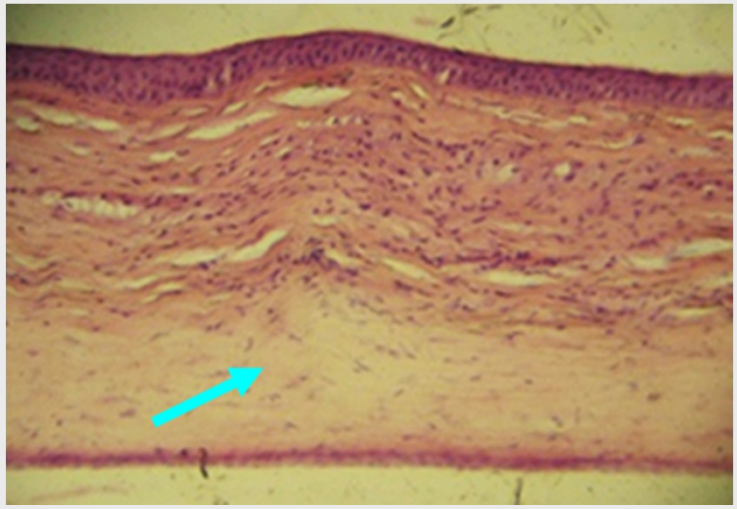


Cryopreserved and lyophilized porcine cornea

Experiments in vivo

- 36 mini-pigs (36 eyes) and 72 rabbits (72 rabbits) were operated on
- operation method: intralamellar implantation and DALK
- both clinical observations and light microscopy showed no cases of graft-versus-host disease in 12 month follow-up in cryo-lyophilized porcine cornea group
- 56% of eyes with transplanted native porcine cornea developed graft-versus-host disease





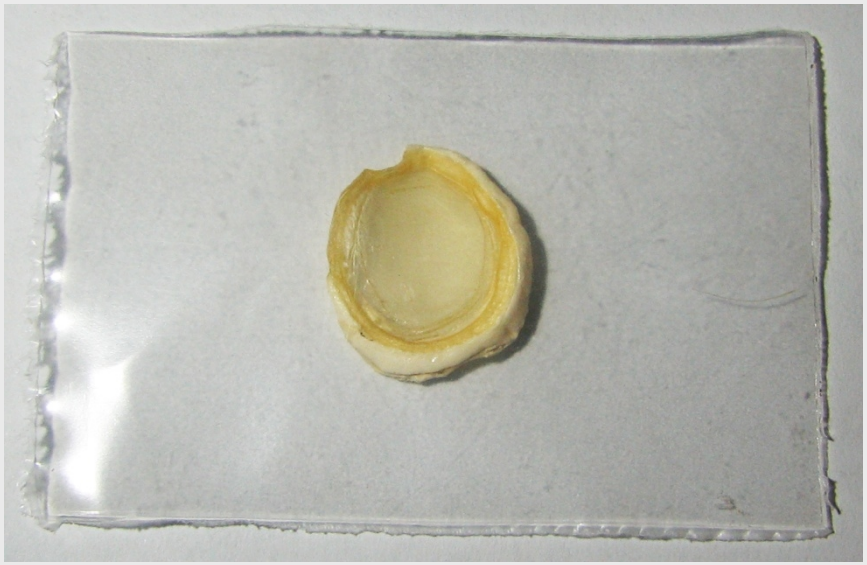
Rabbit eye 12 months after intralamellar cryo-lyophilized porcine cornea transplantation



“Keratoxenoimplant”

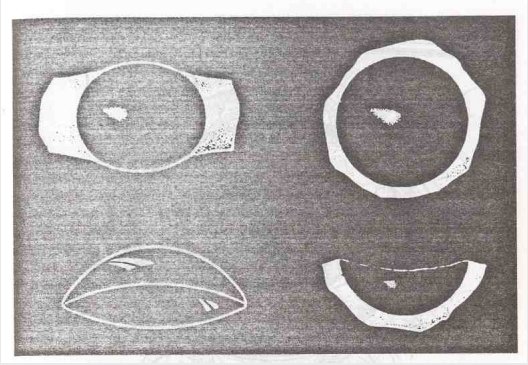


After registration of a medical product and getting approval from State Inspection Board on Drug Quality Control phase I clinical trial was initiated



Clinical trial design

- purpose – to study safety and therapeutic efficiency of cryo-lyophilized porcine cornea transplantation in patients with corneal ulceration
- design - prospective multi-center open-label
- primary endpoint – to save eye globe
- secondary endpoints – complications, visual acuity
- 30 patients (31 eyes):
  - grade IV chemical burn – 20 patients (21 eye, 3 – perforated)
  - infectious keratitis – 10 patients (10 eyes, 2 – perforated)
- follow-up – 12 months
- method – lamellar or full-thickness tectonic corneal transplantation by Puchkovskaia NA * +/- conjunctival/Tenon's flap +/- tarsorrhaphy



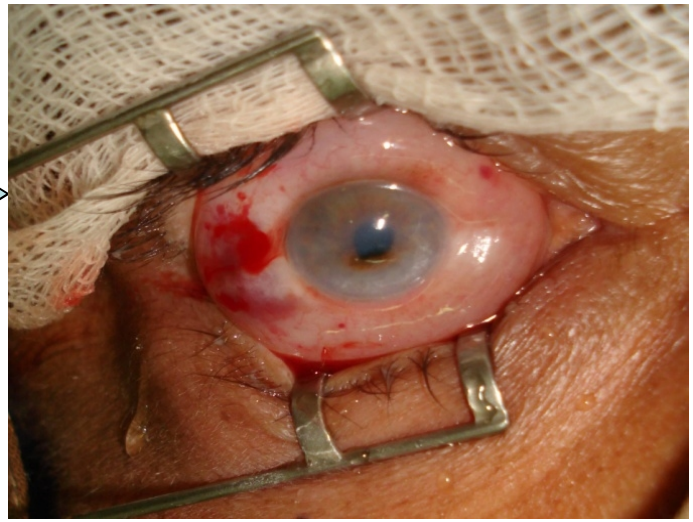
Forms of corneal grafts used for transplantation

* Puchkovskaia NA Therapeutic lamellar keratoplasty. Oftalmol Zh. 1968;23(2):83-7.

Clinical trial results

- all eyes were saved
- graft lysis – 19 patients, xenocornea engraftment (with opacity and vascularisation) – 11 patients
- BCVA ≥0.01 – 12/30 patients
- complications – graft rejection (2 patients with chemical burn)

Perforated bacterial corneal ulcer before

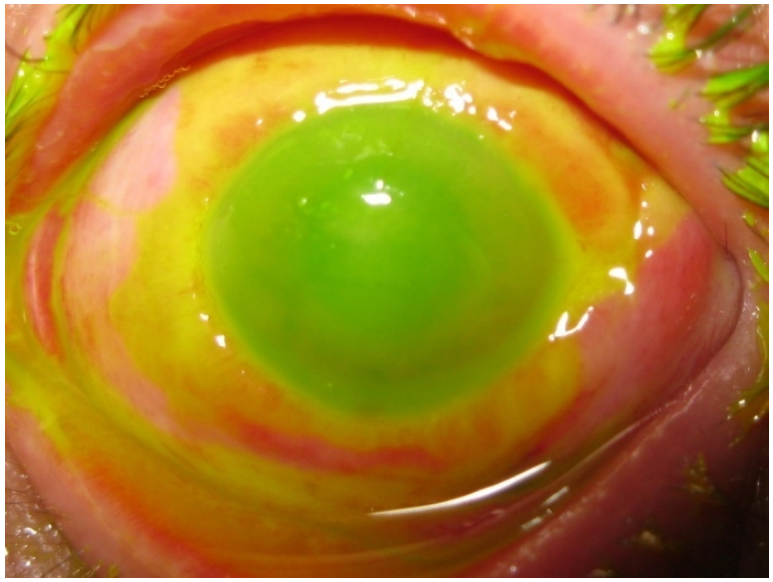




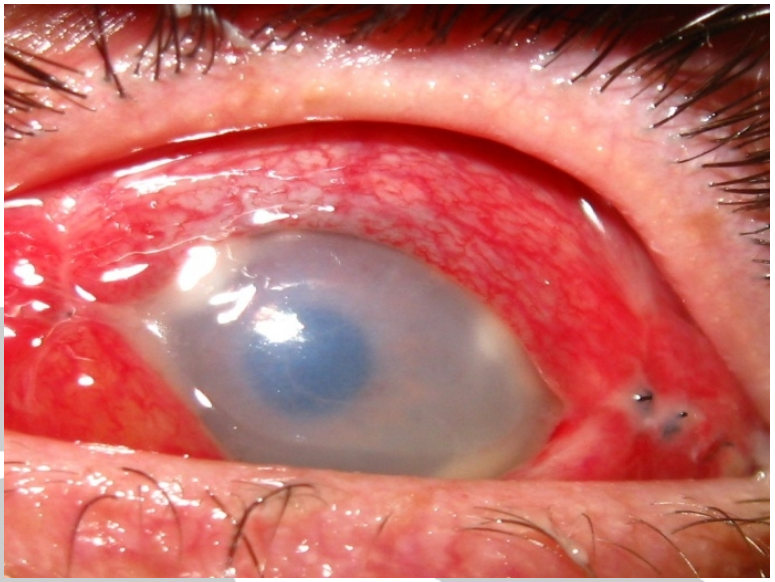
7th day after full-thickness cornea xenotransplantation

12 month follow-up. The graft lysed. Vascularised leukoma. BCVA = 0.02

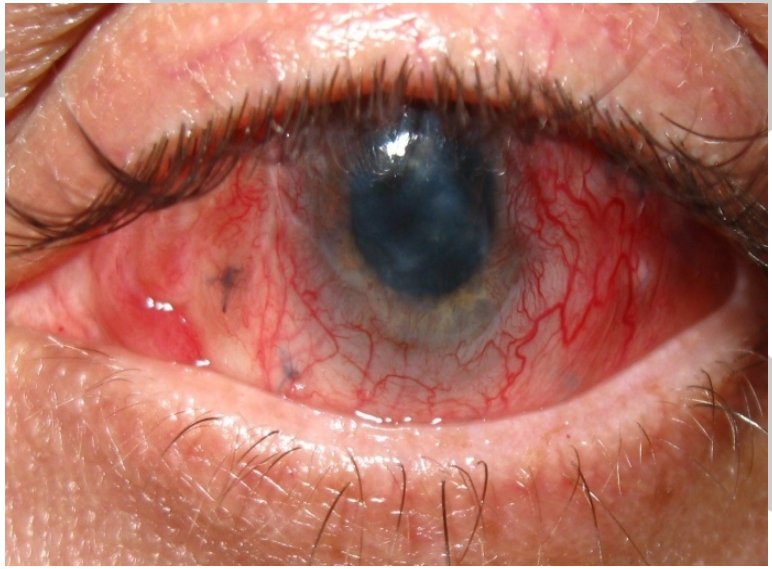




Grade IV chemical burn.
Total corneal ulcer and circular paralimbal conjunctiva necrosis



14th day after lamellar corneal xenotransplantation



12 month follow-up. The graft lysed,
Non-intensive leukoma.
BCVA = 0.12

Cornea xenografting + subsequent K-Pro



Corneal xenotransplantation Conclusion

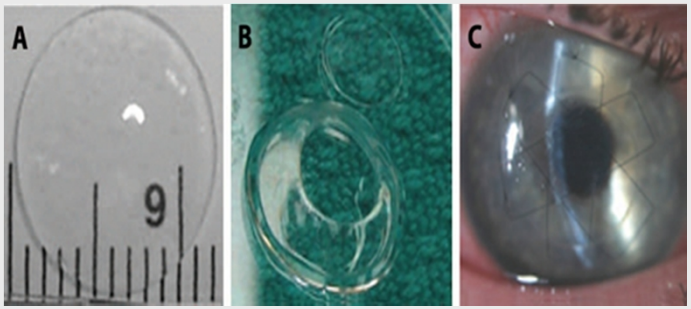
- First results of application of porcine corneal xenografts prepared using developed technology of preservation and storage in corneal ulcer treatment indicate on expedience of further clinical trials
- These xenografts might become cheap and long-term stored corneal substitutes for eye globe rescue if no human donor cornea and amniotic membrane available

III – COLLAGEN-BASED CORNEAL SUBSTITUTES

State-of-art of collagen-based bioengineered corneal substitutes

- **Fabrication technology:**
  - type I (III) collagen-based hydrogel
  - medical grade collagen sources – porcine, fish, human recombinant
  - collagen concentration 10-18%
  - to increase implant strength and biodegradation resistance:
    - collagen cross-linking
    - interpenetrating networks

- **Properties of the fabricated cell-free implants (Griffith M, *et al*, 2003-present):**
  - In vitro studies:
    - physical, chemical and optical properties similar to human cornea
    - collagenase resistant
    - tensile strength – 5-20 times weaker to human cornea
  - Clinical evaluation (experimental animals & phase I clinical trial):
    - stimulated both corneal tissue and nerve regeneration
    - no or low immune reactions without need for immunosuppression

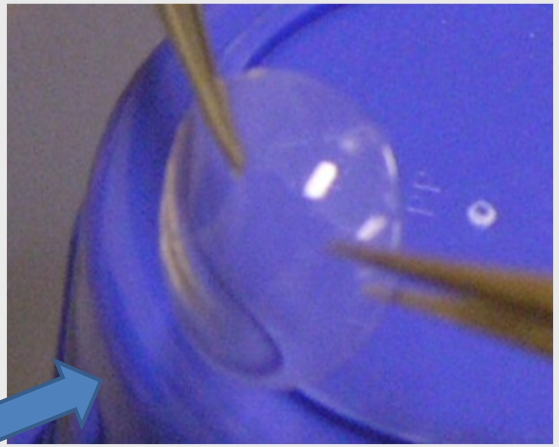


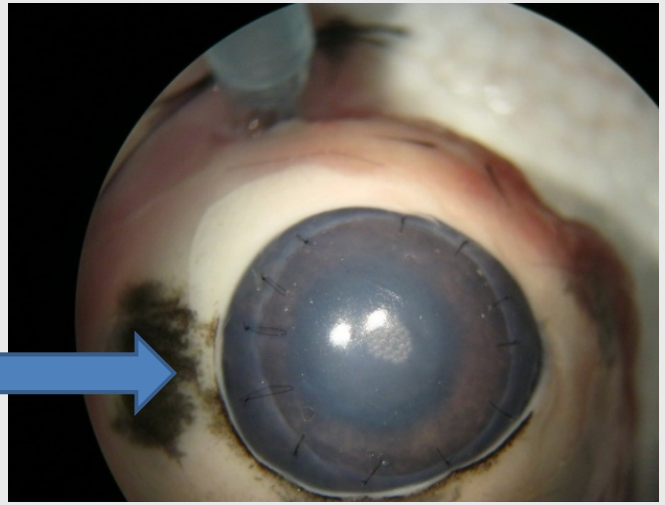
500 μm thick collagen implant 1 month after transplantation in keratoconus patient using DALK technique *

* *P. Fagerholm, et al. A biosynthetic alternative to human donor tissue for inducing corneal regeneration: 24-month follow-up of a phase 1 clinical study. Sci. Transl. Med. 2, 46ra61 (2010).*

Implants fabricated at Filatov Institute

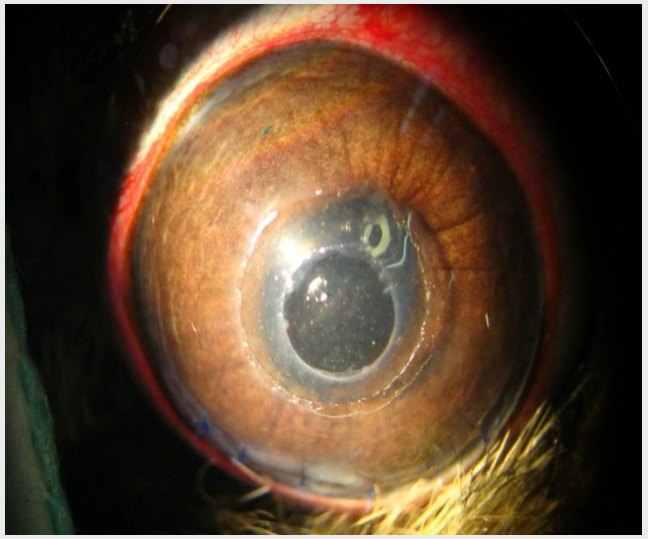
- **Implant fabrication**:
  - porcine type 1 atelocollagen (Koken Co., Ltd, Japan)
  - collagen cross-linking with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide and N-hydroxysuccinimide (Merck & Co., Inc., USA)
  - collagen concentration – 12-16%
- **Implant properties:**
  - almost transparent with single dotty air bubble inclusions
  - 200-500 μm thick
  - refractive indices of 1.24-1.3 (human cornea 1.37-1.38)
  - were elastic and strong enough to tolerate well placement of interrupted stitches during deep lamellar keratoplasty performed on ex vivo porcine eyes



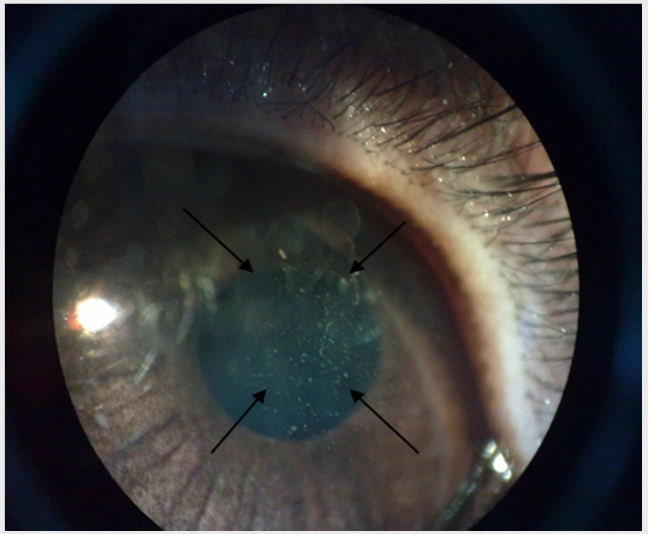


Experiment *in vivo*

- 8 rabbits (8 eyes)
- **“Sutureless” implantation method**
  - a corneal incision 5 mm in length and 4/5 thickness in depth 2 mm from the limbus
  - a 7x7 mm corneal “pocket” was formed with a spatula through this incision
  - the implant was introduced into the corneal “pocket” with use of the spatula
  - corneal incision was closed with 4-6 9/0 nylon sutures
  - the superficial corneal layers over the implant were then removed using 5 mm trephine
  -
- **Clinical evaluation**
  - no adverse inflammatory reactions were observed in all animals
  - epithelial coverage over the implant was completed within five days post-surgery
  - no neovascularisation was observed in the implant location area
  - the implants remained stably integrated and clear during 12 month follow-up



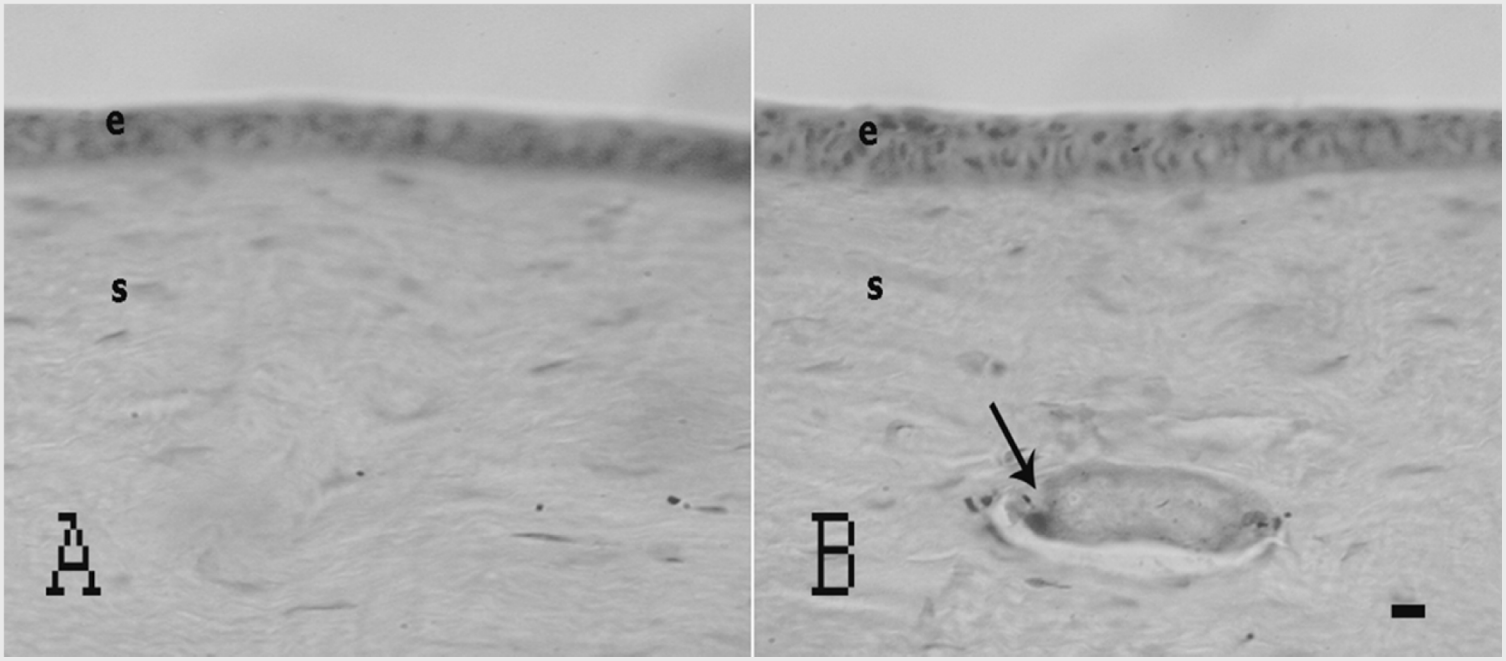
1st day after surgery



12 months after surgery

Experiment *in vivo*

Histopathologic evaluation



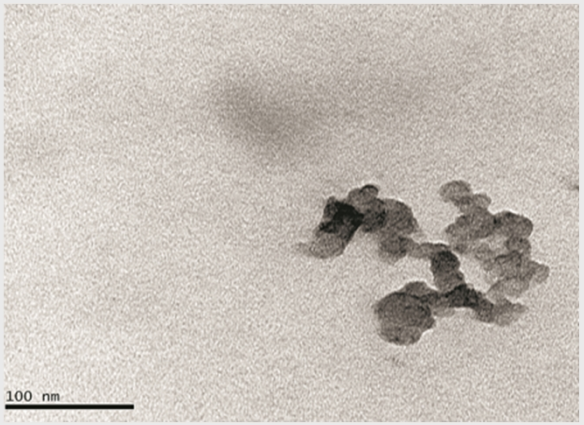
H&E staining of unoperated (A) and operated cornea (B), 12 months after implantation, both showing a stratified epithelium (e) over a stroma (s). A small central piece of still cell-free implant (arrow) lies in reconstituted corneal stroma (B). Scale bar, 50 μm *

** Pasyechnikova N, Vit V, Leus M, Iakymenko S, Buznyk O, et al. Collagen-based bioengineered substitutes of donor corneal allograft implantation: assessment and hypotheses. Med Hypothesis Discov Innov Ophthalmol. 2012 Spring;1(1):10-13.*

Implants with anti-infective peptide sustained delivery system *

- **Purpose -** to develop corneal substitutes (CS) that will serve a dual purpose of:
  - promoting corneal regeneration as an alternative to allograft transplantation
  - delivering anti-infective peptides (AIP) as an alternative to anti-viral drugs / antibiotics from within the CS to treat infection and to prevent new infection
- **Methods:**
  - AIP LL37 (cathelicidin) was encapsulated in silica nanoparticles under magnetic stirring
  - Particles with encapsulated AIP LL37 were then introduced in CS's during their fabrication
  - CS fabrication method: interpenetrating network of type I collagen and 2-methacryloyloxy-ethylphosphorylcholine (MPC) – Liu W, et al (2009)
  - Anti-viral properties of CS were tested against type 1 herpes simplex virus (HSV-1)



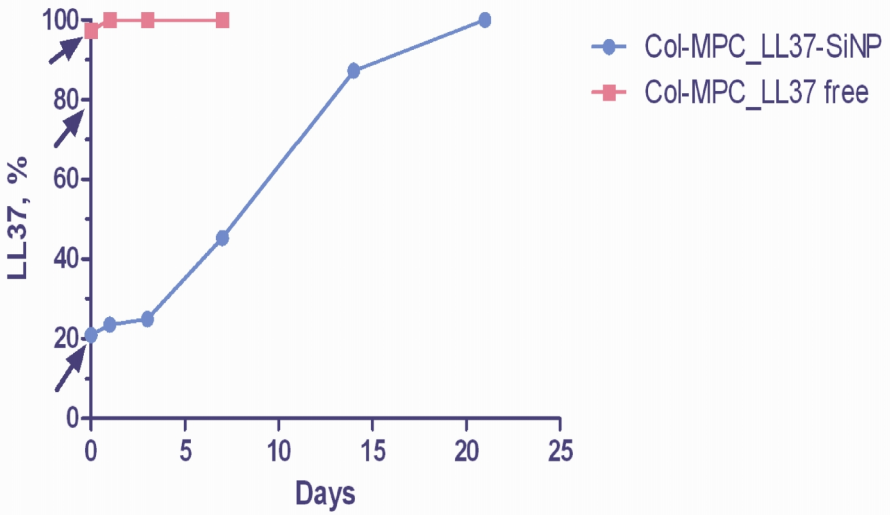


Silica nanoparticles with embedded LL37, particle size 10-20 nm

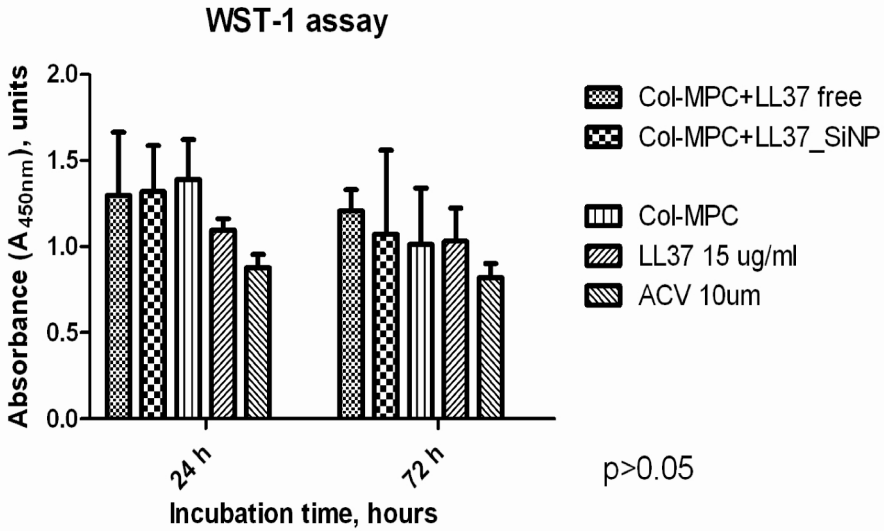
** Lee CJ, Buznyk O, Kuffova L, Rajendran V, Forrester JV, et al. Cathelicidin LL-37 and HSV-1 Corneal Infection: Peptide Versus Gene Therapy. Transl Vis Sci Technol. 2014 May 29;3(3):4.*

Implants with anti-infective peptide sustained delivery system

LL37 release from corneal substitutes



Cytotoxicity study



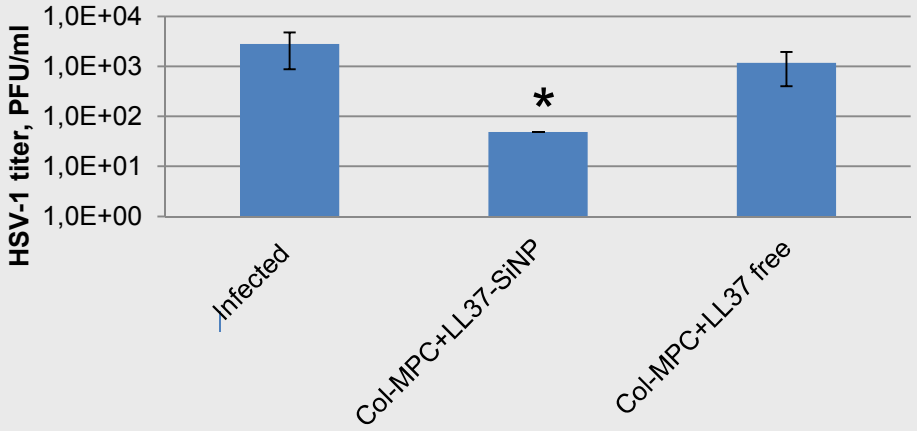
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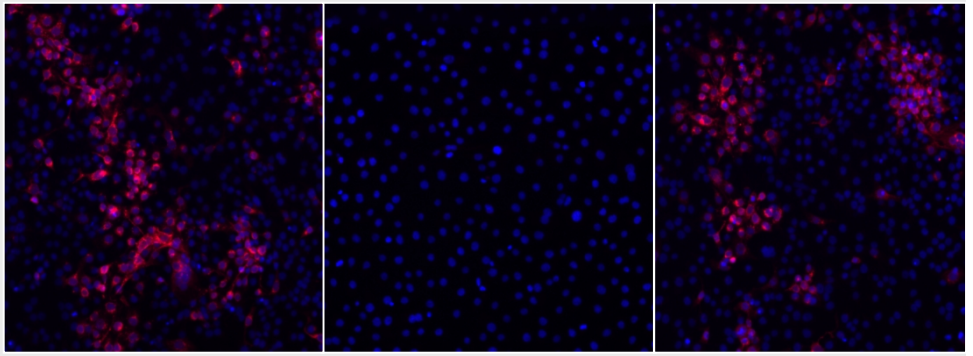
Col-MPC_LL37-SiNP – corneal substitute (CS) with LL37 loaded in silica nanoparticles
Col-MPC_LL37 free – corneal substitute with free L37
Col-MPC – clear corneal substitute
ACV - acyclovir

Implants with anti-infective peptide sustained delivery system

HSV-1 plaque formation assay

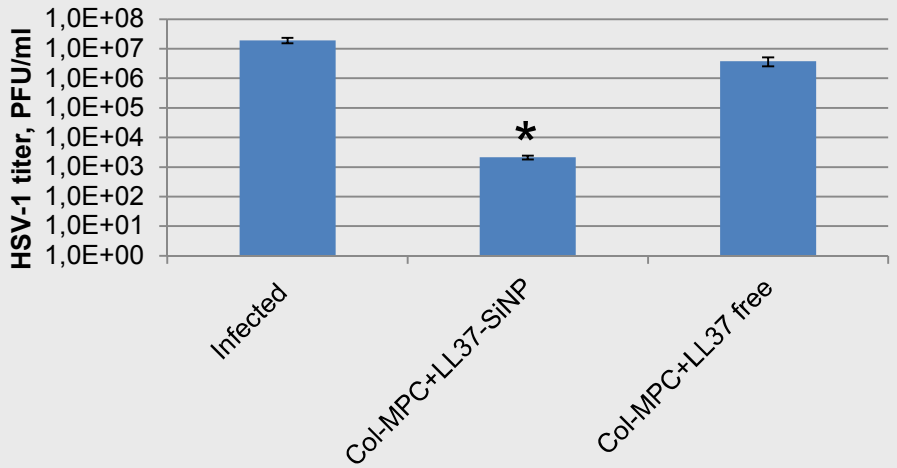
Corneal substitute with silica nanoparticle encapsulated or free LL37 was put on HSV-1 infected human corneal epithelial cells

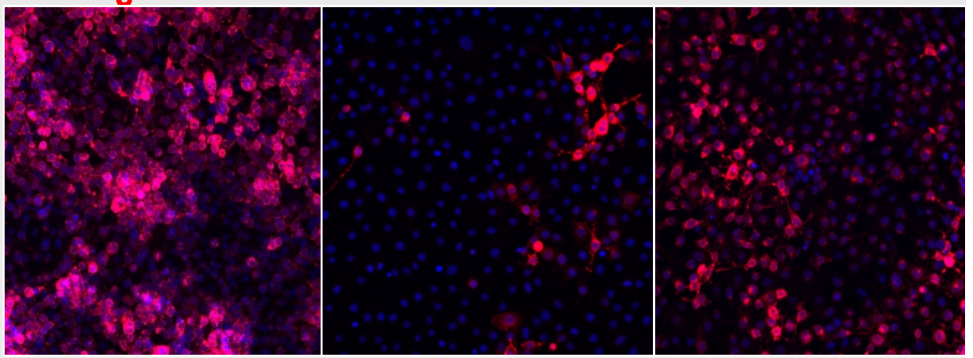




Infected without drug | Col-MPC + LL37-SiNP | Col-MPC + LL37 free

Corneal substitute with silica nanoparticle encapsulated or free LL37 was put on human corneal epithelial cells and co-cultured 24 h prior adding of HSV-1





Infected without drug | Col-MPC + LL37-SiNP | Col-MPC + LL37 free

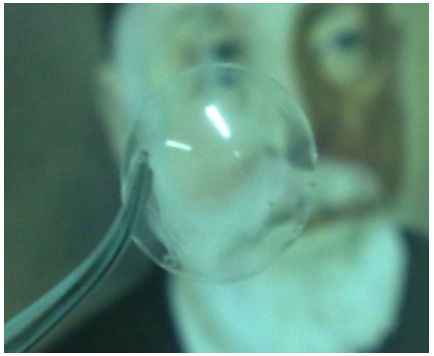
*** p = 0.01 (Bonferroni corrected) compared to infected cells without treatment**

Immunofluorescence Analysis

Notes: Red signal (anti-HSV antibody) – herpes simplex virus infected cell
Blue signal (DAPI) – cell nuclei

Clinical trial

- **Purpose -** to test safety and effectiveness of collagen-MPC corneal substitutes (CS)* in patients with persistent corneal ulcers
- Approval of Filatov Institute Bioethic Committee and State Inspection Board on Drug Quality Control (EudraCT no. 2013-002442-37)
- Design – prospective, hospital based, open-label
- Primary endpoint – corneal ulcer healing
- Secondary endpoints – complications, visual acuity
- 10 patients (10 eyes) will be enrolled (4 already enrolled):
  - post-chemical burn ulcer (3 patients)
  - post-PK ulcer (1 patient)
- Follow-up at the moment – 6-12 months
- Method – anterior lamellar keratoplasty + bandage soft contact lens

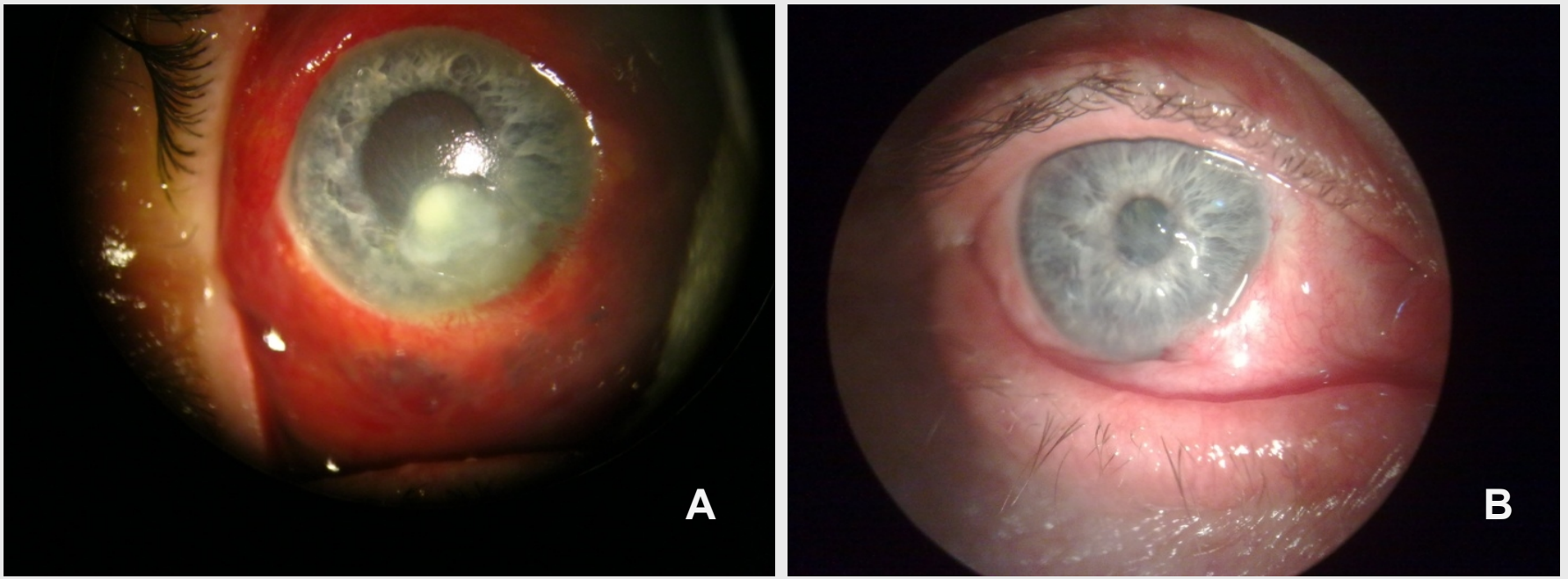


** Hackett JM, Lagali N, Merrett K, Edelhauser H, Sun Y, et al. Biosynthetic corneal implants for replacement of pathologic corneal tissue: performance in a controlled rabbit alkali burn model. Invest Ophthalmol Vis Sci. 2011; 52(2) : 651-657.*

Results

- All implants were well tolerated, no inflammatory reactions were seen
- The implants did not lyse, preserved clarity and epithelialized in 2-8 weeks after the surgery
- Tiny precipitates and haze appeared at the implant posterior surface on the 3rd postoperative week in all cases. They resolved in 2 weeks after topical steroids were prescribed
- Visual acuity improved on 1-2 lines compared to preoperative level in all patients

Results

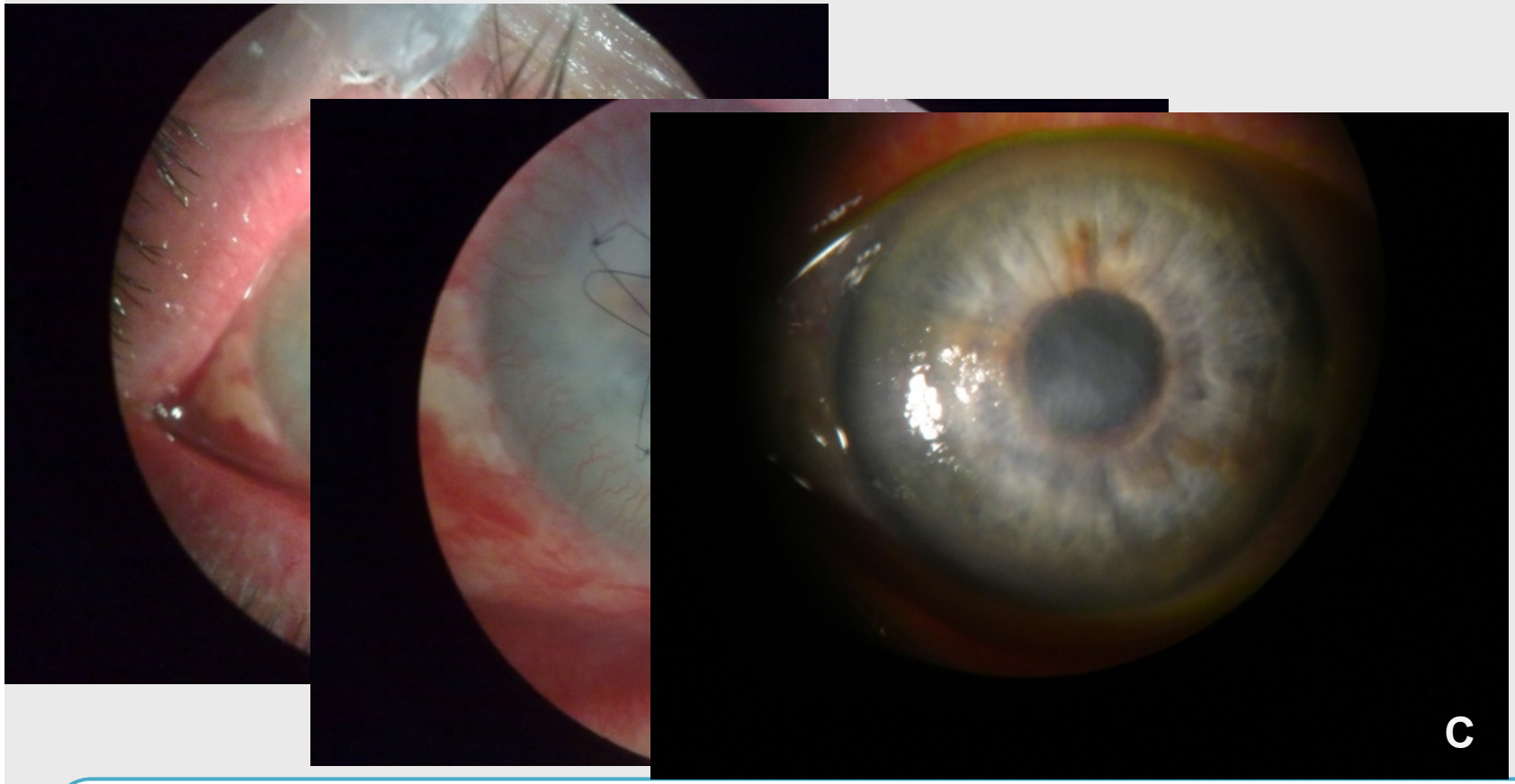


A – Corneal ulcer 2 months after alkali burn, BCVA = 0.04

B – 12 months after CS transplantation (diameter 5 mm, thickness 250 μm). BCVA = 0.12

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Results

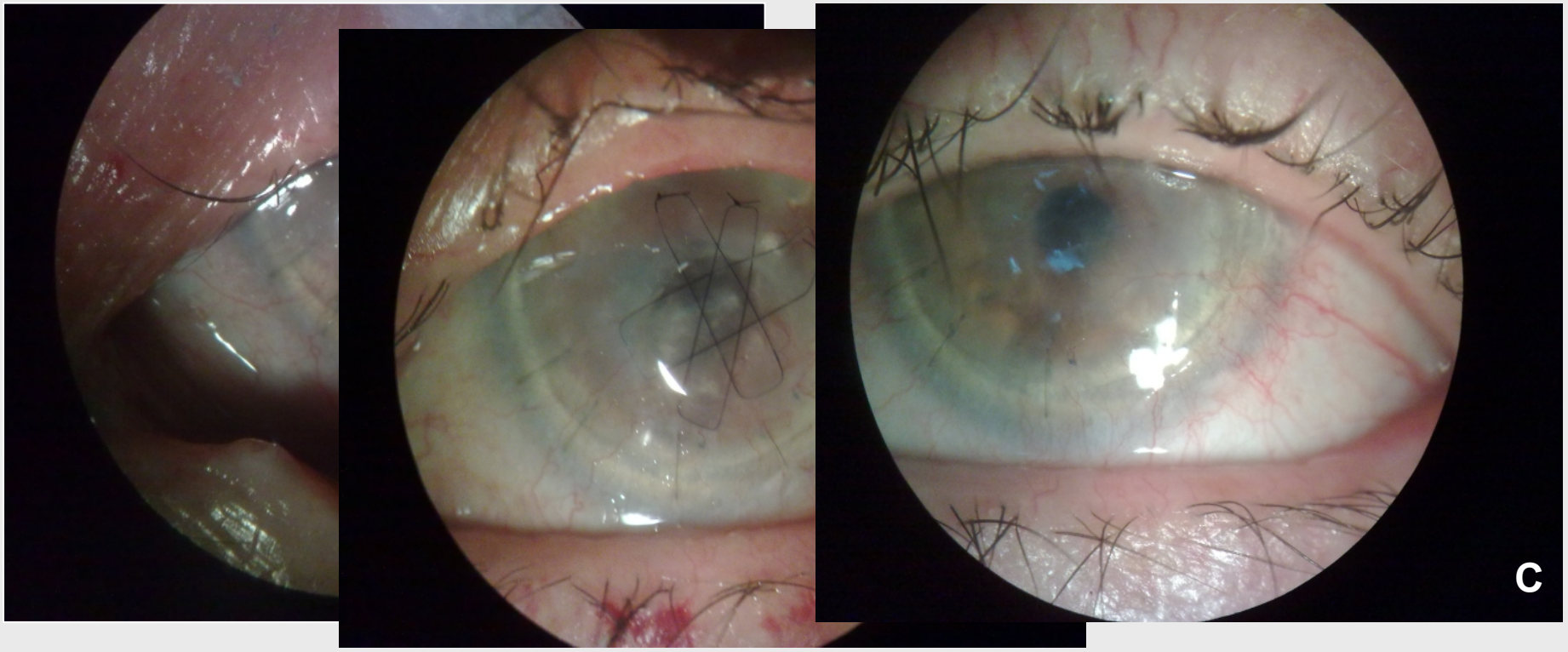


A – Corneal ulcer 2 months after alkali burn, BCVA = 0.02

B – 1 week after CS transplantation Ø 5 mm, 250 µm thick – the implant is transparent, secured with overlying sutures, BCVA 0.03

C – 9 months post-op – non-intensive corneal opacity, BCVA 0.17

Results



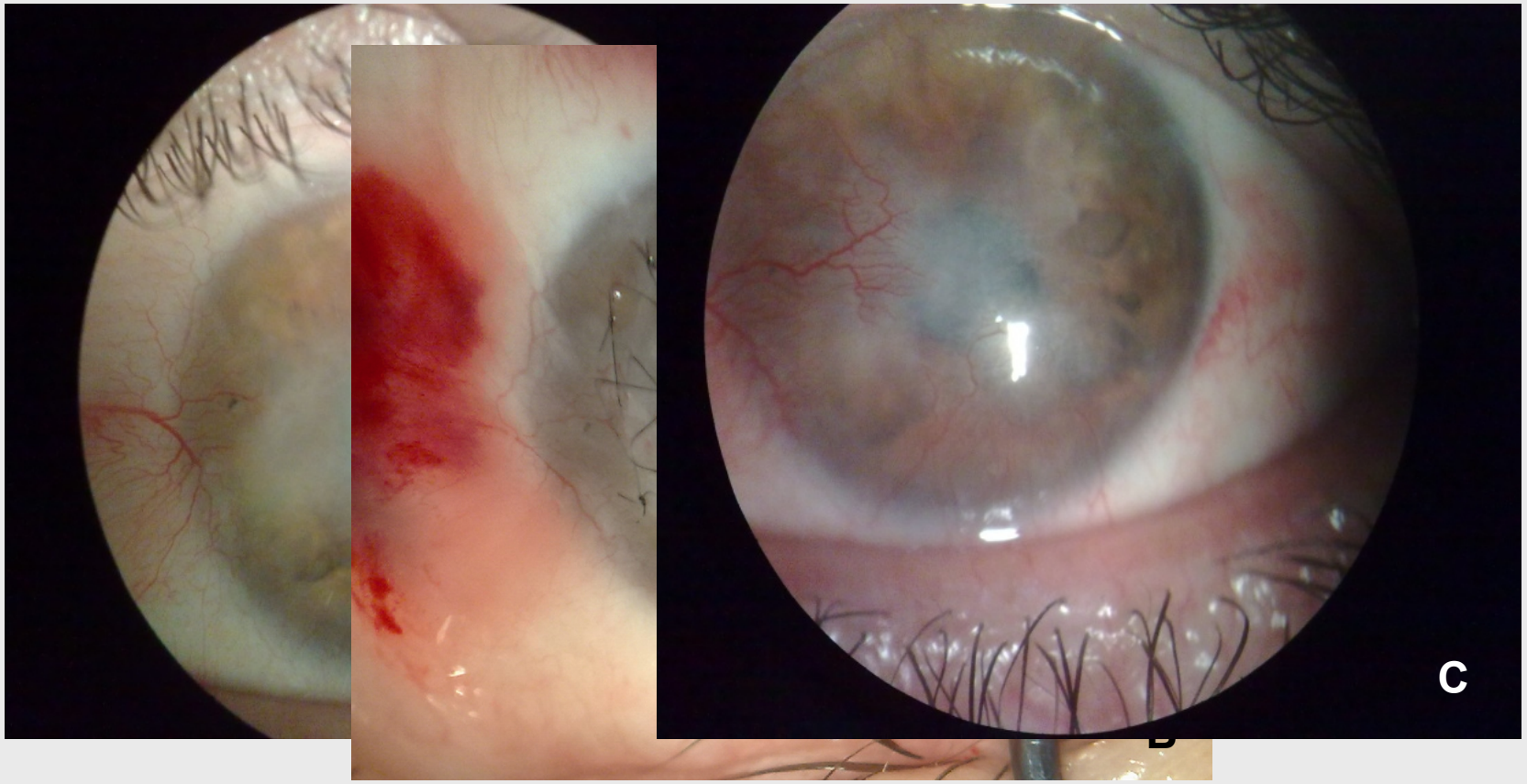
A – corneal ulcer (arrow) 14 months after penetrating corneal graft + cataract extraction + IOL implantation. BCVA = light perception

B – 2 weeks post-op (diameter 4 mm, 250 μm thick) – transparent implant secured with overlying sutures. Corneal haze on the implant posterior surface and near the implant. BCVA 0.01

C – 6 months post-op – transparent implant covered with semi-transparent corneal epithelium, BCVA 0.06

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Results



A – Corneal stroma dystrophy with frequent recurrent erosions 36 months after acid burn, BCVA 0.01
B – at the end of surgery, implant Ø 5 mm, 350 μm thick
C - 6 months after– transparent implant covered with conjunctivalised epithelium. Epithelial surface is stable, no erosion recurrences. BC visual acuity 0.03

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Collagen-based corneal substitutes Conclusion

- First results of collagen-based biosynthetic human corneal stroma clinical application indicate on safety of the developed implants and their ability to withstand corneal ulceration and promote corneal surface restoration in non-infectious corneal ulcer patients
- Further controlled clinical studies needed

Summary

- Due to the increasing worldwide shortage of human donor corneas, keratoprosthesis, cornea xenotransplantation and regenerative medicine strategies using biosynthetic collagen implants developed and are being developed at Filatov Institute allow preserve and restore vision in patients with severe corneal disease

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THANK YOU!



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