Contact Lens and Anterior Eye 47 (2024) 102145

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Case Report

Severe ulcerative keratopathy following implantation of an acellular porcine corneal stromal lenticule in a patient with keratoconus

Tim Berger [a,*], Ursula Schlötzer-Schrehardt [b], Fidelis Flockerzi [c], Loay Daas [a], Elias Flockerzi [a], Berthold Seitz [a]

[a] Department of Ophthalmology, Saarland University Medical Center, Homburg, Saar, Germany
[b] Department of Ophthalmology, University of Erlangen-Nürnberg, Erlangen, Germany
[c] Institute of Pathology, Saarland University Medical Center, Homburg, Saar, Germany

ARTICLE INFO

Keywords:
Cornea
Acellular porcine corneal stroma
Ulcerative keratopathy
Keratoconus
Keratoplasty

ABSTRACT

Purpose: To report a case of ulcerative keratopathy following implantation of acellular porcine corneal stroma (APCS) in a patient with keratoconus (KC).
Methods: A 58 year-old patient initially presented with an ulcerative keratopathy in the left eye. Previously, several corneal procedures (including radial keratotomy, laser-in-situ keratomileusis, crosslinking) were performed for KC. Eight months ago, an APCS lenticule (Xenia corneal implant, Gebauer Medizintechnik GmbH, Neuhausen, Germany) was implanted into a stromal pocket because of progressive keratectasia. Visual acuity was hand movement. Anterior segment optical coherence tomography showed a space between the APCS lenticule and the host stroma. Excimer laser-assisted penetrating keratoplasty (PKP, 8.0/8.1 mm) was performed in the left eye. The corneal explant was investigated by light and transmission electron microscopy.
Results: Best-corrected visual acuity was 20/40 six weeks after PKP. Light microscopy demonstrated a stromal ulceration down to the APCS lenticule. No stromal cells could be found within the APCS lenticule eight months after implantation. The APCS lenticule did not show a green stain of the collagens with Masson-Goldner staining and exhibited a strong Periodic acid-Schiff positive reaction. Electron microscopy of the APCS lenticule revealed cross-linked collagen lamellae without cellular components. Close to the interface, corneal collagen lamellae of the host cornea were disorganized. Few vital keratocytes were present on the surface of the lenticule and appeared to cause mechanical disruption of the host stroma along the lenticule-stroma interface.
Conclusion: APCS implantation may lead to severe complications such as ulcerative keratopathy in otherwise uncomplicated KC corneas. In such cases, excimer laser-assisted PKP or Deep Anterior Lamellar Keratoplasty are the methods of choice to restore visual acuity.

1. Introduction

Corneal allograft transplantation is an established procedure to treat corneal blindness for more than 100 years [1]. As the demand for human donor corneas far exceeds the supply in certain regions of the world, research is focusing on new sources of corneas for clinical transplantation [2,3]. However, the clinical experience with alternative grafting options such as xenotransplantation (e.g. pig cornea-to-human) and bioengineered corneas (3D-bioprinting) is limited and requires further investigation to solve the imbalance between supply and demand [4–8].

Xenotransplantation is a promising option in many areas of medicine to address organ shortages and might be a particularly attractive approach in the cornea due to the immune privileged status with a relatively low risk of graft rejection compared to other organ transplants [7,9,10]. A variation of corneal xenotransplantation is the use of an acellular porcine corneal stroma (APCS) lenticule, which is processed from porcine corneas by removing nuclear and cellular components to achieve a low antigenicity as keratocytes constitute from 3 % to 5 % of the natural stromal volume [11]. In general, the APCS lenticule is primarily used as a substitute for emergency lamellar keratoplasty (APCS-LK), whereas only one study to date has reported clinical and functional outcomes following the implantation of APCS to stabilize thinned keratoconus (KC) corneas [12].

* Corresponding author.
E-mail address: tim.berger@uks.eu (T. Berger).

https://doi.org/10.1016/j.clae.2024.102145
Received 21 November 2023; Received in revised form 4 March 2024; Accepted 9 March 2024
Available online 11 March 2024

This case report is the first to present a patient with severe ulcerative keratopathy after APCS lenticule implantation who required excimer laser-assisted penetrating keratoplasty (PKP), and provides light and electron microscopic findings of the corneal explant.

2. Case report

2.1. Patient history and clinical presentation

A 58-year-old male patient underwent several refractive procedures for myopia correction due to KC in the left eye, including radial keratotomy (25 years ago) and laser-in-situ keratomileusis (LASIK, 10 years ago). Due to progressive keratectasia, corneal crosslinking (CXL, four years ago) and repeat LASIK combined with CXL (three years ago) were performed.

Eight months ago, the patient underwent an APCS lenticule implantation at an external clinic to stabilize the severely thinned cornea of the left eye as keratectasia progressed.

In Germany, the stromal lenticule is commercially available (Xenia corneal implant, Gebauer Medizintechnik GmbH, Neuhausen, Germany) and derived from a porcine cornea that underwent a decellularization process to completely remove the cellular components containing antigens. The tissue has also been stabilized by a CXL process *ex vivo*. Surgery was performed under topical anesthesia and the porcine lenticule (*ex vivo* thickness: 100 µm, diameter: 8 mm) was implanted in the host cornea after creating an intrastromal pocket at 40 % of the corneal thickness using a femtosecond laser.

At the initial presentation of the patient eight months after APCS implantation, the left eye (Fig. 1**A-C**) showed melting of the overlying host stroma with a severe corneal ulcer down to the APCS lenticule. The corneal defect was restricted to the stromal area above the lenticule. There were no clinical signs of infection. Corneal neovascularization was present in the radial keratotomy incisions. The clinical findings in the right eye were normal without tomographic or biomechanical evidence of KC. Visual acuity was hand movement in the left eye and 20/20 in the right eye.

Anterior segment optical coherence tomography (AS-OCT) (Fig. 2) demonstrated a space at the horizontal interface between the APCS lenticule and the underlying host stroma. Besides, the axial power map exhibited a severe irregularity of the anterior corneal surface of the left eye.

An excimer laser-assisted PKP (8.0/8.1 mm) was performed on the left eye due to the severe findings. The postoperative course was uneventful. At six weeks follow-up, the graft was completely epithelialized and clear (Fig. 1**D**). Best-corrected visual acuity was 20/40 in the affected eye six weeks after PKP.

2.2. Microscopic examination

The light microscopic examination (Fig. 3) demonstrated a corneal ulceration of the host cornea extending to the APCS lenticule. In contrast to the surrounding host stroma, the APCS lenticule did not show a green stain of the collagens with Masson-Goldner staining but exhibited a strong Periodic acid-Schiff (PAS) positive reaction. The latter method indicated a carbohydrate-rich tissue. No cells could be found within the APCS lenticule. Vimentin-positive cells appeared at the interface between the APCS lenticule and the host stroma, suggestive of fibroblastic transformation. The lenticule thickness as well as the thinnest part of the host stroma were both 50 µm. A mild cellular inflammatory infiltrate was present in the host stroma.

Electron microscopy (Fig. 4) of the APCS lenticule showed crosslinked collagen lamellae without cellular debris. Close to the interface, corneal collagen lamellae of the host cornea were disorganized and amorphous extracellular material was dispersed between the lamellae. The keratocytes were partially degenerated in the host stroma. Few vital keratocytes or fibroblasts were present on the surface of the lenticule and appeared to cause mechanical disruption of the host stroma along the lenticule-stroma interface.

3. Discussion

Since 2015, APCS has been used clinically as a substitute for a human cornea in therapeutic lamellar keratoplasty to treat various corneal infectious diseases. The surgical steps of therapeutic APCS-LK include a partial trephination, lamellar keratectomy of the diseased tissue, and fixation of the APCS lenticule with interrupted single sutures analogous

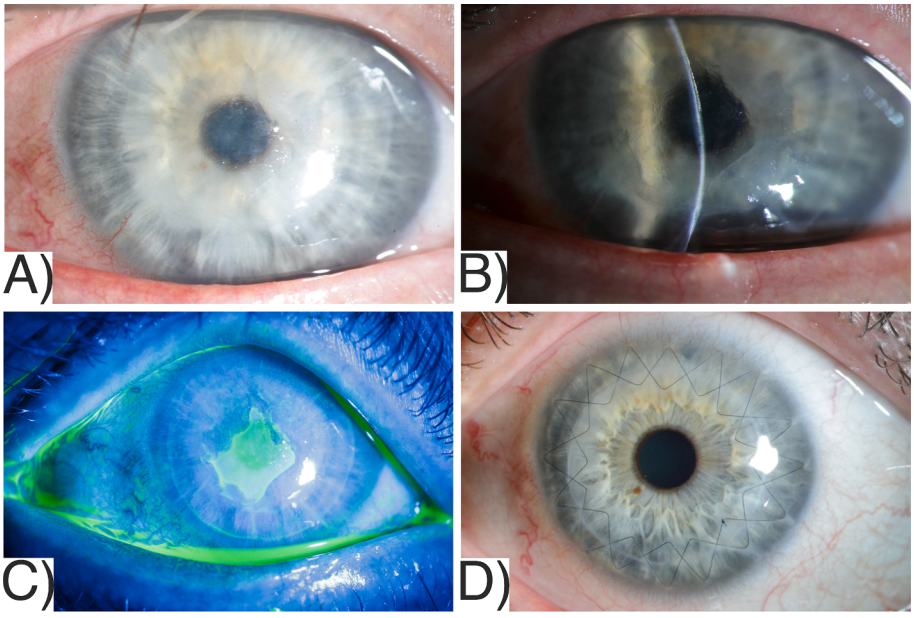

Fig. 1. Pre- and postoperative slit-lamp biomicroscopic photographs. A-C) The preoperative photographs demonstrate a central corneal ulcer extending to the acellular porcine corneal stromal (APCS) lenticule with melting of the overlying host stroma by diffuse (A) and thin slit illumination (B). Fluorescein vital staining (C) marks the extent of the central ulceration. **D)** Postoperative findings six weeks after excimer laser-assisted penetrating keratoplasty (8.0/8.1 mm) show a clear graft that was fixed with a double running suture according to Hoffmann.

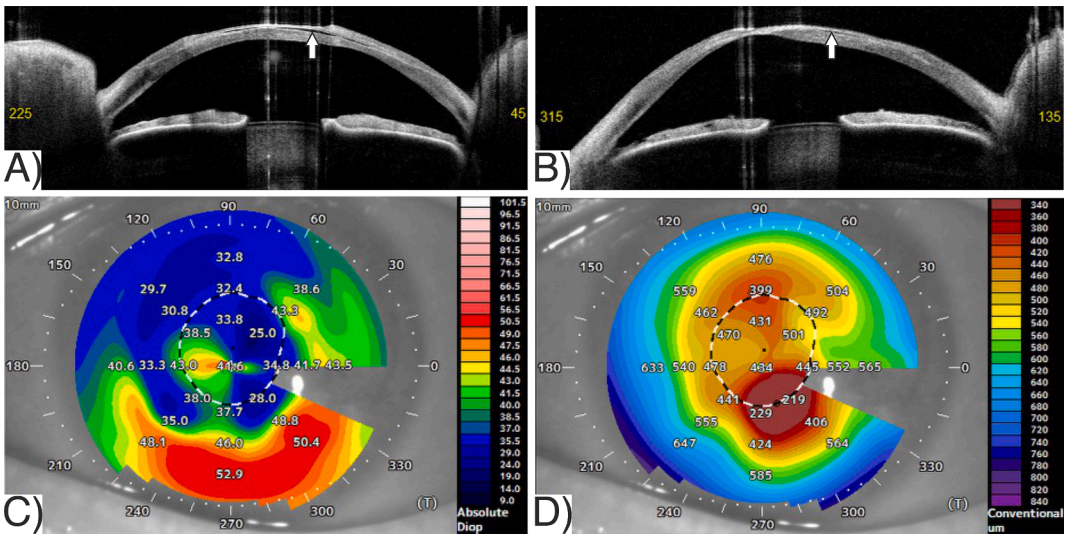

Fig. 2. Preoperative anterior segment optical coherence tomography (AS-OCT). A, B) The sections are showing a central ulceration of the host stroma down to the acellular porcine corneal stroma (APCS) lenticule. The host cornea is severely thinned (B). A dehiscence between the APCS lenticule and the underlying host stroma is marked by arrows. **C)** The axial power map is demonstrating a severe irregularity with inferior steepening and superior flattening of the anterior corneal surface. **D)** The pachymetry map displays a paracentral thinning of the cornea with a thinnest point of 219 μm.

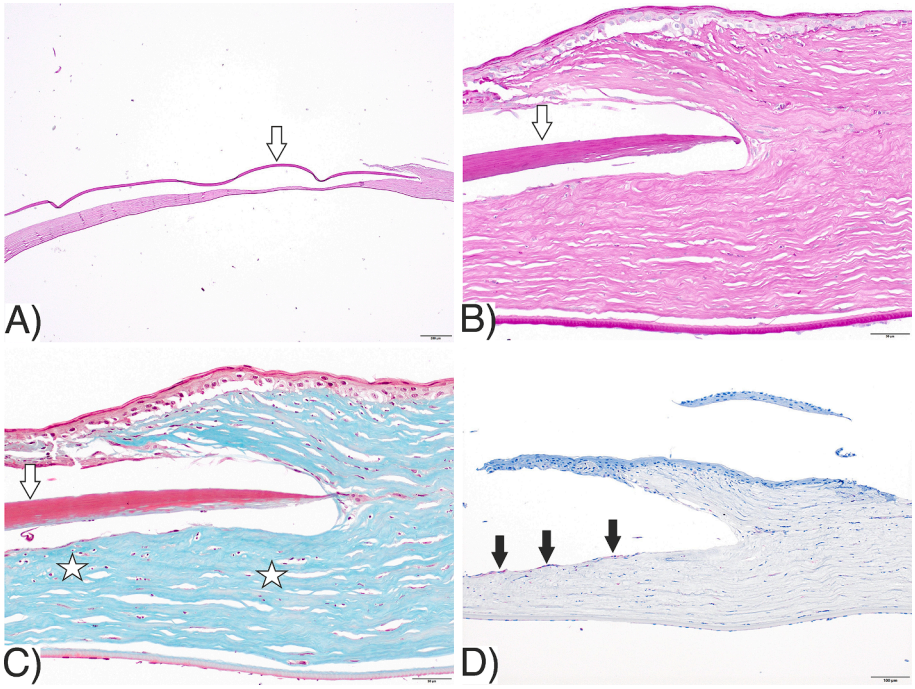

Fig. 3. Light microscopy of the corneal explant. A, B) The host cornea is demonstrating a stromal ulceration down to the acellular porcine corneal stroma (APCS) lenticule (white arrow), which exhibits a strong Periodic acid-Schiff (PAS) positive reaction. No stromal cells can be found within the APCS lenticule eight months after implantation. The thickness of the lenticule and the thinnest part of the host cornea measure 50 μm. Periodic acid-Schiff (PAS)-reaction, original magnification 2× (A), 20× (B). **C)** In contrast to the surrounding host stroma, the stromal lenticule does not show a green stain with Masson-Goldner staining. A mild cellular inflammatory infiltrate of the host cornea (stars) is visible near the lenticule by using a higher magnification. Masson-Goldner stain, original magnification 20 ×. **D)** Vimentin-positive cells (black arrows) appear at the interface between the APCS lenticule (not shown) and the host stroma, suggestive of fibroblastic transformation. Vimentin immunostaining, original magnification 10 ×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to human corneal tissue [11,13–16].

The use of APCS in elective procedures has only been described in one study for stabilization of thinned KC corneas [12]. In contrast to therapeutic APCS-LK, the lenticule is implanted into a femtosecond laser-created intrastromal pocket. The purpose is to improve visual acuity by altering the corneal structure, as in advanced stages of keratectasia, more invasive procedures such as Deep Anterior Lamellar Keratoplasty (DALK) or PKP are the only remaining surgical options [17]. Nevertheless, little is known about potential risks associated with APCS implantation in ectatic corneas, as no complications have ever been reported in the literature.

For the first time, a case of severe ulcerative keratopathy as a

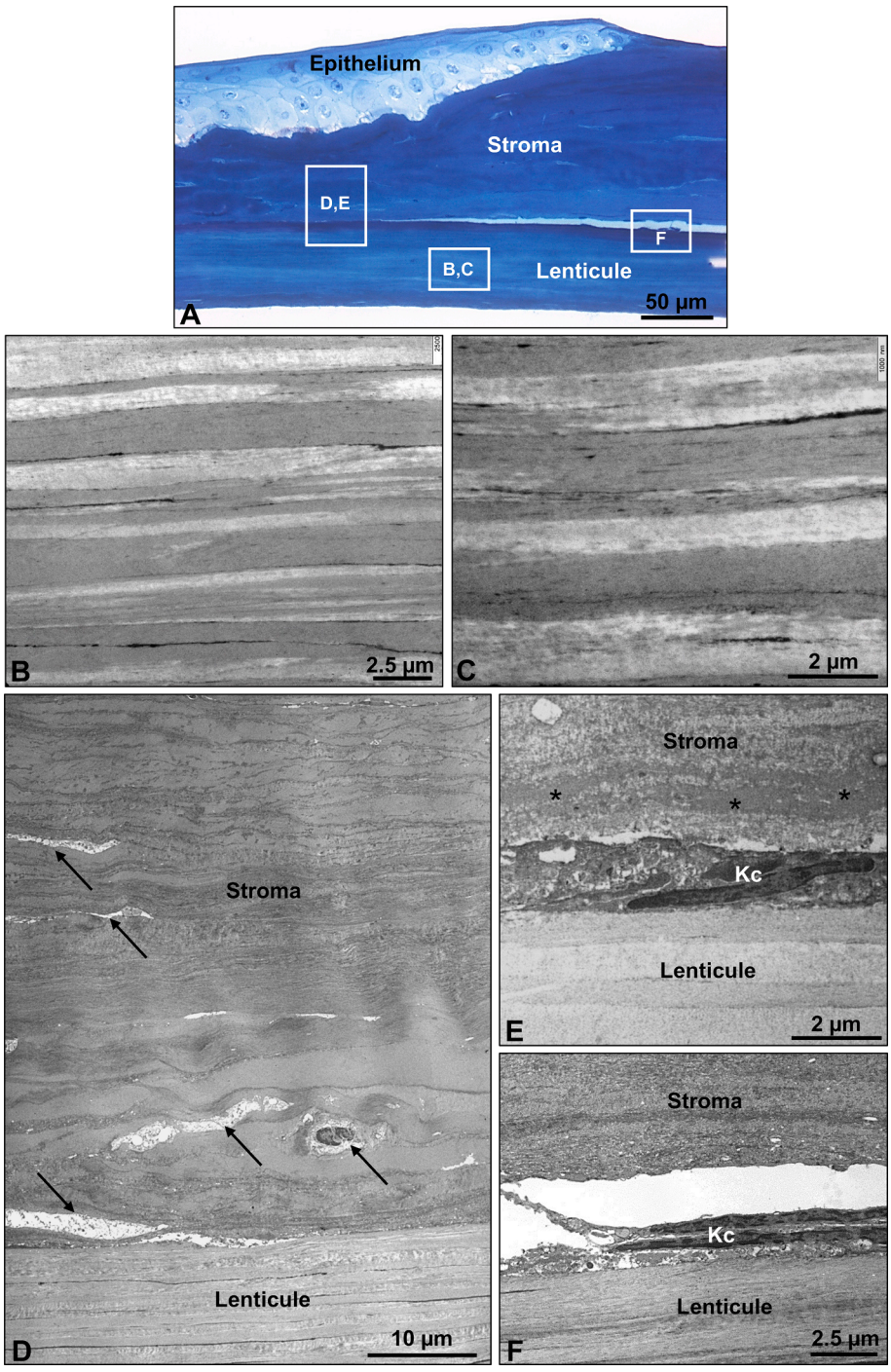

Fig. 4. Transmission electron microscopy of the corneal explant. A) Semithin section showing localization of the lenticule underneath the host corneal epithelium and stroma. Boxes indicate localization of electron micrographs (B-F). **B, C)** Electron micrographs of the lenticule consisting of cross-linked collagen lamellae without any remaining cells. **D)** Ultrastructure of the host corneal stroma adjacent to the lenticule. Close to the interface, the stromal collagen lamellae appear disorganized and the keratocytes (arrows) are degenerated. **E)** Electron micrograph showing the interface between lenticule and host stroma. Keratocytes (Kc) line the surface of the lenticule. Amorphous extracellular material (asterisks) is dispersed between the stromal collagen lamellae. **F)** Strong adherence of keratocytes or fibroblasts to the lenticular surface may cause mechanical disruption of the host stroma along lenticule-stroma interfaces.

complication after implantation of an APCS lenticule in KC is presented together with light and electron microscopy of the corneal explant.

The only study to date examined the outcome after APCS lenticule implantation in 20 KC patients without contact lens tolerance of two clinical cohorts (India and Iran) over a 24-month period [12]. No postoperative complications were reported in all 20 patients [12].

Although clinical experience with APCS is very limited, there have been several reports of complications following therapeutic APCS-LK. These complications included reduced graft transparency of varying severity, corneal neovascularization, and early suture loosening [11,13,14,16]. It is important to note that in almost all cases, the lenticule never became clear and more or less severe corneal graft opacities persisted even months after the procedure, which might not be satisfying to any corneal surgeon. Complete corneal clarity, as can be achieved after PKP, was not present.

Li et al. reported graft calcification after therapeutic APCS-LK in

three of 85 patients (3.5 %) after one month postoperatively [15]. Furthermore, one study found a high rate of graft melting in three of 13 patients (23.1 %) after APCS-LK in herpetic keratitis [14].

In the present case, implantation of an APCS lenticule resulted in corneal melting of the overlying host stroma. The nutrition of the cornea with glucose is mainly provided by the aqueous humor with increasing concentrations from the posterior to the anterior stroma [18]. The structurally and chemically altered lenticule might have disturbed the diffusion of metabolites, causing malnutrition and melting of the overlying host stroma. Furthermore, unlike the adjacent host stroma, light microscopy of the APCS lenticule showed a strong PAS reaction and no green staining of the collagens with Masson-Goldner stain, which could also indicate that the collagens were no more present in their natural form. The pronounced PAS positivity could be attributed to glycogen being used as a possible crosslinking agent, however, nothing is known about the exact preparation of the lenticule.

Another microscopic finding is the absence of stromal cell migration in the lenticule even eight months after implantation, which was confirmed by multiple histologic sections through the lenticule. Li et al. demonstrated by in vivo confocal microscopy that stromal cell repopulation of APCS grafts did not occur until three months after surgery. Even after 12 months, only a small number of stromal cells were present in the graft [11]. In contrast, Shi et al. found a large number of stromal cells in APCS grafts after two months [19]. The reduced stromal cell migration might also indicate why the lenticule showed dehiscence towards the host stroma, since fibrotic remodeling for graft integration did not occur, and stromal cells were seen only sporadically on the lenticule surface, except for Vimentin-positive cells at the interface of the host stroma.

This case illustrates that implantation of an APCS lenticule might lead to severe complications such as ulcerative keratopathy in otherwise uncomplicated KC corneas. In such cases, excimer laser-assisted PKP or DALK are the methods of choice to restore visual acuity [1,17,20].

CRediT authorship contribution statement

Tim Berger: Conceptualization, Writing – original draft. **Ursula Schlötzer-Schrehardt:** Methodology, Writing – review & editing. **Fidelis Flockerzi:** Methodology, Writing – review & editing. **Loay Daas:**Writing – review & editing. **Elias Flockerzi:** Writing – review & editing. **Berthold Seitz:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Seitz B, Szentmáry N, El-Husseiny M, Viestenz A, Langenbucher A, Naumann GOH. The Penetrating Keratoplasty (PKP): A Century of Success. In: Hjortdal J, editor. Corneal Transplantation. Cham, Switzerland: Springer International Publishing; 2016. p. 67–92. https://doi.org/10.1007/978-3-319-24052-7_6.
[2] Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, et al. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol 2016;134:167–73. https://doi.org/10.1001/jamaophthalmol.2015.4776.
[3] Flockerzi E, Turner C, Seitz B, Collaborators GSG. Descemet's membrane endothelial keratoplasty is the predominant keratoplasty procedure in Germany since 2016: a report of the DOG-section cornea and its keratoplasty registry. Br J Ophthalmol 2023. epub ahead of print. doi: 10.1136/bjo-2022-323162.
[4] Lamm V, Hara H, Mammen A, Dhaliwal D, Cooper DKC. Corneal blindness and xenotransplantation. Xenotransplantation 2014;21:99–114. https://doi.org/10.1111/xen.12082.
[5] Kim MK, Wee WR, Park CG, Kim SJ. Xenocorneal transplantation. Curr Opin Organ Transplant 2011;16:231–6. https://doi.org/10.1097/MOT.0b013e328344870c.
[6] Hara H, Cooper DKC. The immunology of corneal xenotransplantation: a review of the literature. Xenotransplantation 2010;17:338–49. https://doi.org/10.1111/j.1399-3089.2010.00608.x.
[7] Yoon CH, Choi HJ, Kim MK. Corneal xenotransplantation: where are we standing? Prog Retin Eye Res 2021;80:100876. https://doi.org/10.1016/j.preteyeres.2020.100876.
[8] Hara H, Cooper DKC. Xenotransplantation-the future of corneal transplantation? Cornea 2011;30:371–8. https://doi.org/10.1097/ICO.0b013e3181f237ef.
[9] Cooper DKC, Gaston R, Eckhoff D, Ladowski J, Yamamoto T, Wang L, et al. Xenotransplantation - the current status and prospects. Br Med Bull 2018;125:5–14. https://doi.org/10.1093/bmb/ldx043.
[10] Wang Y, Lei T, Wei L, Du S, Girani L, Deng S. Xenotransplantation in China: present status. Xenotransplantation 2019;26:1–8. https://doi.org/10.1111/xen.12490.
[11] Li S, Deng Y, Tian B, Huang H, Zhang H, Yang R, et al. Healing characteristics of acellular porcine corneal stroma following therapeutic keratoplasty. Xenotransplantation 2020;27:1–8. https://doi.org/10.1111/xen.12566.
[12] Rafat M, Jabbarvand M, Sharma N, Xeroudaki M, Tabe S, Omrani R, et al. Bioengineered corneal tissue for minimally invasive vision restoration in advanced keratoconus in two clinical cohorts. Nat Biotechnol 2023;41:70–81. https://doi.org/10.1038/s41587-022-01408-w.
[13] Zhang MC, Liu X, Jin Y, Jiang DL, Wei XS, Xie HT. Lamellar keratoplasty treatment of fungal corneal ulcers with acellular porcine corneal stroma. Am J Transplant 2015;15:1068–75. https://doi.org/10.1111/ajt.13096.
[14] Zheng J, Huang X, Zhang Y, Wang Y, Qin Q, Lin L, et al. Short-term results of acellular porcine corneal stroma keratoplasty for herpes simplex keratitis. Xenotransplantation 2019;26:1–9. https://doi.org/10.1111/xen.12509.
[15] Li H, Dong M, Zhou Q, Zhao L, Wang F, Wang X, et al. Corneal calcification of acellular porcine corneal stroma following lamellar keratoplasty. Acta Ophthalmol 2022;100:164–74. https://doi.org/10.1111/aos.14665.
[16] Shi W, Zhou Q, Gao H, Li S, Dong M, Wang T, et al. Protectively Decellularized porcine cornea versus human donor cornea for Lamellar transplantation. Adv Funct Mater 2019;29:1902491. https://doi.org/10.1002/adfm.201902491.
[17] Daas L, Hamon L, Flockerzi E, Suffo S, Seitz B. Excimer laser-assisted keratoplasty: penetrating keratoplasty "Excimer-PKP" and deep anterior lamellar keratoplasty "Excimer-DALK". In: Alió JL, del Barrio JLA, editors. Modern Keratoplasty. Essentials in Ophthalmology. Cham, Switzerland: Springer International Publishing; 2023. p. 159–66. https://doi.org/10.1007/978-3-031-32408-6_11.
[18] Reim M, Lax F, Lichte H, Turss R. Steady state levels of glucose in the different layers of the cornea, aqueous humor, blood and tears in vivo. Ophthalmologica 1967;154:39–50. https://doi.org/10.1159/000305147.
[19] Shi Y, Bikkuzin T, Song Z, Jin X, Jin H, Li X, et al. Comprehensive evaluation of decellularized porcine corneal after clinical transplantation. Xenotransplantation 2017;24:1–7. https://doi.org/10.1111/xen.12338.
[20] Seitz B, Langenbucher A, Kus MM, Küchle M, Naumann GO. Nonmechanical corneal trephination with the excimer laser improves outcome after penetrating keratoplasty. Ophthalmology 1999;106:1156–65. https://doi.org/10.1016/S0161-6420(99)90265-8.