DOI: 10.15825/1995-1191-2026-1-154-163

CORNEAL XENOTRANSPLANTATION IN OPHTHALMOLOGY: CHALLENGES AND PROSPECTS

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Introduction. This paper evaluates the key structural characteristics of turkey cornea and critically analyzes both the obtained results and existing literature to assess the feasibility of using this xenogeneic material for keratoplasty. Currently, more than 12 million patients worldwide are awaiting keratoplasty. The prolonged waiting times is largely driven by severe shortage of cadaveric human corneas required for the procedure. Recent studies have generated growing optimism regarding the potential of xenogeneic keratoplasty; however, the number of animal donors studied to date remains quite limited. In light of this gap, the objective of this study was formulated. **Objective:** to assess the main structural characteristics of turkey (*Meleagris gallopavo*) cornea and to evaluate its potential use as a xenogeneic material for selective keratoplasty. **Materials and methods.** Corneoscleral buttons (n = 24) were isolated from enucleated turkey eyeballs. In the first stage, the corneal microstructure was examined using scanning electron microscopy and confocal microscopy. In the second stage, the feasibility of cutting out a corneal xenograft suitable for deep anterior lamellar keratoplasty was evaluated. In the third stage, the potential for preservation of the obtained xenografts using xenogeneic turkey corneal material was assessed. **Results.** The turkey cornea was shown to possess all the principal layers characteristic of the human cornea. Its mean thickness was 508 ± 33.5 μm, and the presence of Bowman's membrane was confirmed. Preservation of the turkey cornea for up to five days was feasible while maintaining a high endothelial cell density. In addition, the preparation of a xenograft suitable for deep Anterior lamellar keratoplasty from turkey corneal tissue was successfully demonstrated. **Conclusion.** Analysis using confocal microscopy and scanning electron microscopy, together with assessment of xenograft integrity following hypothermic preservation, indicates that turkey cornea represents a promising xenogeneic material for selective keratoplasty. Further studies are warranted to assess its potential application in reconstructive corneal surgery.

Keywords: cornea, turkey, keratoplasty, xenotransplantation, xenogeneic material.

INTRODUCTION

According to recent estimates, there are currently 36 million blind people worldwide [1]. Corneal damage is one of the common causes of vision loss. Currently, restoration of damaged corneal tissue is primarily achieved through keratoplasty – a surgical procedure in which the affected, opacified portion of the recipient's cornea is replaced with a transparent cadaveric donor cornea.

Despite its effectiveness, keratoplasty faces a substantial global demand, with waiting lists exceeding 12 million patients [2, 3]. The primary reason for this shortage is the limited availability of cadaveric donor corneas, which remain the gold standard for transplantation. Consequently, there is growing research interest in the development of alternative graft materials, particularly xenogeneic sources suitable for keratoplasty [4–6].

Historically, the concept of corneal transplantation originated with xenografts. Carl Himly is credited with proposing the idea of xenotransplantation, and his student, Franz Reisinger, performed the first successful corneal transplant in animal experiments in 1818, coining the term "keratoplasty", which remains in use today [7].

The first clinical corneal xenotransplantation – from an animal to a human – was performed by Richard Kissam in 1838 using porcine corneas as donor material [8]. Subsequent experimental attempts involving corneas from various animal species produced limited success. Among these, the most favorable outcomes were reported with gibbon corneal grafts, which remained transparent in approximately 50% of cases over a follow-up period exceeding five months [4].

However, the widespread use of primate-derived xenografts has been constrained by several factors. These include their limited reproductive capacity, which restricts the availability of donor material, as well as the potential risk of transmitting zoonotic infections, including viruses closely related to those affecting humans. In addition, ethical concerns regarding the use of species with close behavioral and biological similarities to humans further limit their acceptability.

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In contrast, pigs offer several practical and biological advantages over non-human primates, making them a more promising source of xenogeneic material for corneal transplantation [4]. Pigs are widely considered a promising source of donor tissue due to several practical advantages. They are relatively easy and cost-effective to breed, have high reproductive rates with large litter sizes, and can be genetically modified to overcome immunological barriers. In addition, pigs can be raised under controlled, pathogen-free conditions. Ethical concerns are also generally less pronounced, as pigs are commonly used for food in many cultures [9].

However, in regions where Islam or Judaism is practiced, the use of porcine-derived materials may be unacceptable on religious grounds [9]. Moreover, the porcine cornea differs from the human cornea in key morphological characteristics. For instance, its thickness varies considerably depending on the breed, typically ranging from 660 to 990 μm, whereas the normal human corneal thickness is approximately 550–560 μm. Since the cornea is a biological lens, its physical properties are of paramount importance. In addition, porcine cornea differs significantly from human cornea. For instance, in pigs, corneal thickness in the central optical zone is approximately equal to that at the periphery, whereas in humans, thickness gradually increases from the center toward the periphery.

Beyond these morphological differences, the porcine cornea is not suitable for humans because it can elicit a strong immune response in humans, limiting its suitability for transplantation. For example, Song et al. reported that the posterior epithelial layer of the porcine cornea expresses co-stimulatory molecules CD80 and CD86, which activate T-cell–mediated immune responses, leading to graft opacification and rejection [10]. Similarly, Islam et al. demonstrated that native porcine cornea induces the production of multiple pro-inflammatory mediators, including interleukins (IL-1β, IL-2, IL-6, IL-8, IL-1ra), chemokines (MCP-1, MIP-1α, MIP-1β), and other cytokines (TNF, G-CSF, IFN-γ, FGF-basic). The authors suggested that combined C5 blockade with eculizumab and TLR4 inhibition may offer therapeutic potential in porcine-to-human corneal xenotransplantation [11].

Another critical concern is the risk of zoonotic infection. Porcine endogenous retrovirus (PERV) remains the most significant viral agent of concern. PERV is capable of integrating into the porcine genome and has been shown to infect human cells *in vitro*. However, to date, there is no conclusive *in vivo* evidence demonstrating transmission of PERV to human recipients [10].

Due to the marked morphological and immunological incompatibility between porcine and human corneas, recent research has increasingly focused on the use of decellularization techniques to prepare corneal xenografts. This approach enables the complete or partial removal of cellular and immunogenic components while preserving the native extracellular matrix and its micro- and macrostructural integrity.

Decellularized corneal tissue can function as an *in vivo* scaffold that supports tissue regeneration while maintaining the physiological properties required for corneal replacement. For clinical application, however, an additional sterilization step is necessary to minimize the risk of transplant-associated infections. In this context, gamma irradiation – an established and widely standardized sterilization method – is commonly employed [12].

In recent years, advances in corneal decellularization have made it possible to overcome major immunological barriers to xenotransplantation, enabling the use of animal-derived corneas in the treatment of human corneal diseases. For instance, in 2019, Zheng et al. used similar decellularized porcine xenografts to treat the complications of keratitis and corneal perforations associated with herpes simplex virus infection [13]. Subsequently, in 2020, the same group reported the successful application of similar grafts in the treatment of fungal keratitis, achieving significant improvements in visual acuity [14].

These findings highlight the effectiveness of decellularization techniques and underscore the growing potential of corneal xenotransplantation, driven in part by advances in the processing and preparation of xenogeneic materials [14].

Advances in corneal xenotransplantation have stimulated comparative studies aimed at identifying the most suitable xenograft sources. For example, Liu et al. (2016) reported the results of an *in vivo* experimental study comparing the efficacy of ostrich and porcine corneal xenografts transplanted into rabbits [15]. Their findings demonstrated significantly higher rates of transparent graft engraftment with ostrich corneas compared to porcine corneas. In discussing these results, the authors emphasized the importance of close morphological and histological compatibility between donor xenograft tissue and the recipient cornea. Based on their analysis, they concluded that the human cornea more closely resembles the ostrich cornea than the porcine cornea in key structural parameters.

The high therapeutic potential of interspecies xenotransplantation had already been demonstrated in earlier studies conducted in Russia in the mid-20th century. For example, in 1956, Professor Arkady Pavlovich Nesterov, in his dissertation "Experimental Studies on Heterogeneous Corneal Transplantation", was the first to demonstrate that avian corneal xenografts – specifically from chickens – yielded the most favorable outcomes in xenokeratoplasty compared with corneas from other animal species [16]. However, due to significant discrepancies in diameter and thickness between the chicken and human corneas, this material is not suitable for contemporary clinical keratoplasty.

In light of these findings, and based on both Nesterov's work [16] and current scientific literature, Sergey. Borzenok has proposed the turkey cornea as a potentially

promising xenograft source for xenokeratoplasty. At present, there is no global consensus regarding the optimal animal donor for corneal xenotransplantation. Although most studies have focused on porcine corneas, substantial differences in anatomical, topographical, morphological, and refractive properties compared with the human cornea remain key limitations to their clinical application.

Accordingly, the objective of the present study was formulated to address this gap.

Objective: to assess the main structural characteristics and suitability of the turkey (*Meleagris gallopavo*) cornea as a potential xenogeneic material for selective keratoplasty.

MATERIALS AND METHODS

To achieve this objective, 12 turkey heads (*Meleagris gallopavo*) were used, from which 24 corneoscleral complexes were isolated following a standard protocol for harvesting cadaveric donor corneas at an eye tissue bank. In the first stage of the study, intact turkey heads were subjected to confocal microscopy to assess corneal thickness, morphological features, and cellular composition. Confocal imaging was performed using the contact method with an HRT3 confocal microscope (Heidelberg Engineering GmbH, Germany). To further evaluate the microstructural characteristics of the turkey cornea, scanning electron microscopy (SEM) was conducted using a JCM-6000 Plus scanning electron microscope (JEOL, Japan). To this end, corneal samples were fixed in a 4% formaldehyde solution for 24 hours. Dehydration was then carried out using a graded series of acetone solutions as follows: 5% acetone for 20 minutes, 10% acetone for 20 minutes, 30% acetone for 20 minutes, 50% acetone for 20 minutes, 70% acetone for 20 minutes, 90% acetone for 20 minutes, and finally 100% acetone (three changes, 20 minutes each). The dehydrated samples were placed in CPD800A microporous capsules (Quorum Technologies Ltd., UK) and subjected to critical point drying for 30 minutes at 31 °C and 7.39 MPa using a K850 drying unit (Quorum Technologies Ltd., UK), during which the acetone was replaced by liquid CO_2. Subsequently, the samples were mounted on aluminum stubs using carbon tape and conductive adhesive, with the endothelial surface oriented upward. They were then transferred to a Smart Coater vacuum sputtering system (Structure Probe, Inc., USA) and coated with a 5 nm layer of gold (99.9% purity) to provide surface conductivity. Following preparation, the samples were examined using a scanning electron microscope to analyze the microstructural characteristics of the corneal tissue.

In the second stage of the procedure, grafts were prepared from the corneoscleral complexes for deep anterior lamellar keratoplasty (ALK). The excised tissue, consisting of the cornea and a portion of the sclera, was oriented with the corneal endothelium facing upward and secured in this position within the Moria ALK Artificial Anterior Chamber (MORIA Inc., France). Normotony was established, after which baseline pachymetry of the central corneal thickness was performed using the iVue100 optical coherence tomograph (Optovue, USA).

Subsequently, the Moria Evolution 3E microkeratome (MORIA Inc., France) was used to obtain a lamellar graft of the desired thickness (approximately 350 µm). A final pachymetric measurement of the residual corneal tissue was then carried out to confirm the thickness and adequacy of the prepared graft for ALK.

In the third stage, the prepared grafts for ALK were placed in vials containing Borzenko–Moroz corneal preservation solution and stored under hypothermic conditions (+4 °C). After 24 hours, graft thickness was evaluated using optical coherence tomography (OCT).

The feasibility of long-term preservation of the xenograft was then assessed using xenogeneic turkey corneal tissue. At the end of the 5-day preservation period, final pachymetric measurements were performed with an optical coherence tomograph. Subsequently, the grafts were removed from the experiment and examined using SEM.

RESULTS

Confocal microscopy of the turkey eye revealed that the cornea comprises all the principal layers characteristic of the human cornea. The presence of Bowman's membrane was confirmed at a depth of approximately 50 µm. This layer exhibits hyperreflectivity and an amorphous structure similar to that of the human Bowman's membrane; however, unlike the human cornea, a subepithelial (subbasal) nerve plexus was not observed on its anterior surface.

The stromal layer of the turkey cornea displayed a distinct cross-striated pattern. In the deeper stromal regions, isolated intrastromal nerve fibers were identified. Compared with the human cornea, the turkey cornea showed a considerably lower density and number of both stromal nerve fibers and keratocytes.

It was also observed that corneal thickness increases gradually and uniformly from the central to the peripheral regions. The presence of Descemet's membrane was confirmed. The endothelial layer consists of a monolayer of hexagonal cells with hyperreflective cytoplasm and hyporeflective intercellular borders. Based on confocal microscopy measurements, the endothelial cell density was determined to be 4373 ± 532 cells/mm^2.

An example of corneal thickness measurement obtained via confocal microscopy is presented in Fig. 1, while the key morphometric parameters of the native turkey cornea are summarized in Table.

SEM revealed that the stromal layer of the turkey cornea is composed of densely packed collagen fibers, with minimal intercellular spaces. Protein strands were observed interconnecting the collagen fibers in the transverse direction. Electron microscopic analysis of the posterior (endothelial) layer showed a monolayer of hexagonal cells characterized by high cellular density and tight intercellular junctions.

Table

Main morphometric characteristics of native turkey cornea

Parameter	Value
Corneal thickness, μm	508 ± 33.5
Stromal thickness, μm	440 ± 29.7
Keratocyte density, cells/mm^2	478 ± 43
Endothelial cell density, cells/mm^2	4373 ± 532

An example of a scanning electron micrograph of the turkey cornea is presented in Fig. 2.

Corneoscleral complexes were harvested from enucleated turkey eyeballs using a standard protocol for cadaveric donor cornea procurement. The harvesting process is illustrated in Fig. 3.

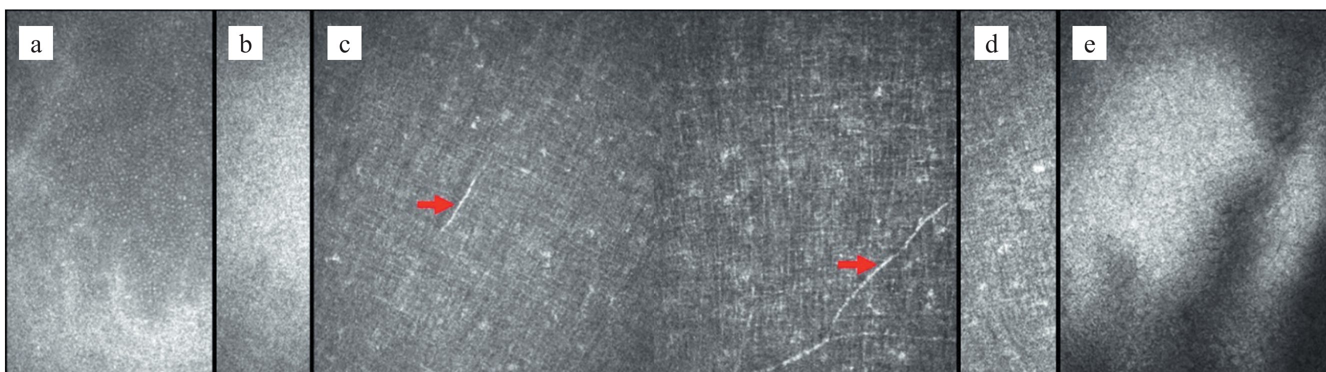

Fig. 1. Confocal microscopy of a turkey cornea: (a) anterior epithelium; (b) Bowman's layer; (c) stroma; (d) Descemet's membrane; (e) posterior epithelium. Arrows indicate stromal nerve fibers

Fig. 2. Scanning electron microscopy of a turkey cornea: (a) overview of the stroma; (b) stromal collagen fibers; (c) overview of the posterior epithelium; (d) hexagonal cells of the posterior epithelium

Grafts intended for ALK were subsequently prepared from the isolated corneoscleral complexes. The mean thickness of the prepared xenografts was 325 ± 42.9 μm. Dissection of the xenograft from the endothelial side, combined with the use of a 130-μm blade, enabled more accurate prediction of the final graft thickness. After 24 hours of preservation, graft thickness increased significantly to 385 ± 53.9 μm.

An example of graft assessment using OCT after one day of hypothermic storage in a corneal preservation solution, with a measured central corneal thickness of 369 μm, is shown in Fig. 4.

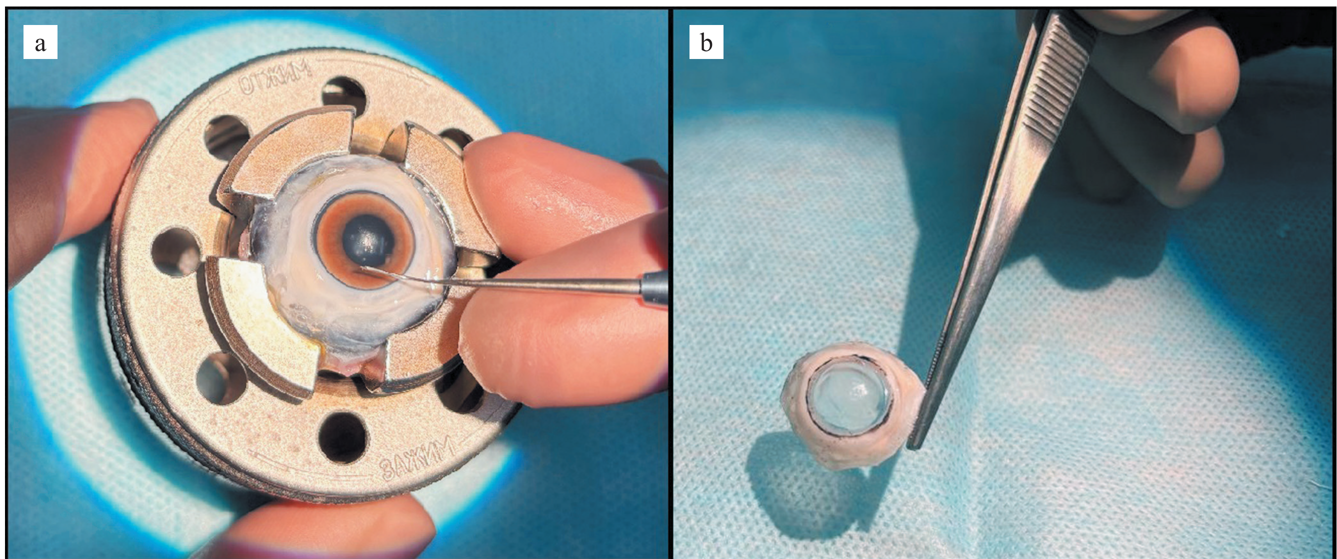

Fig. 3. Procedure for isolating the corneal button (b) from an enucleated turkey eyeball (a)

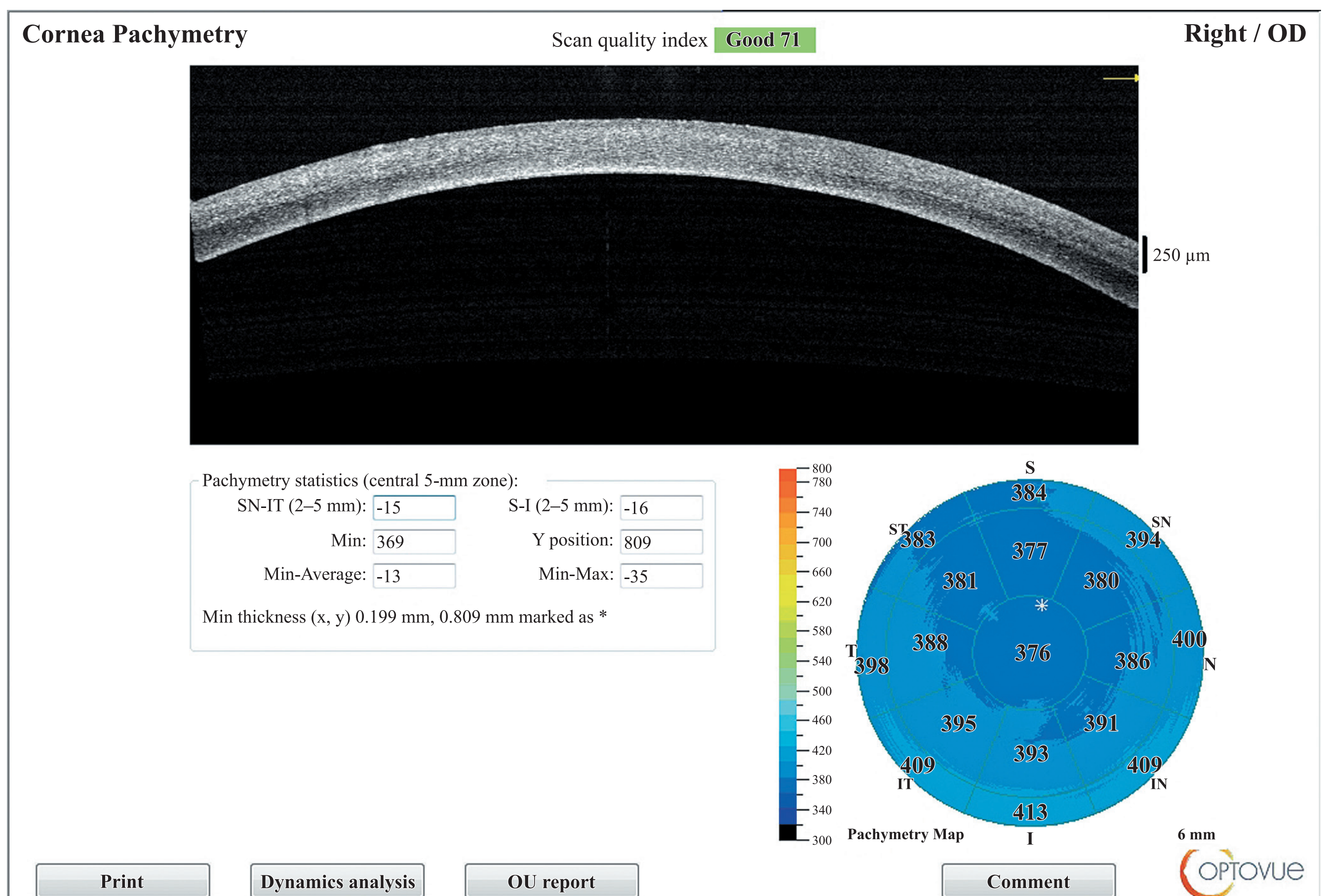

Fig. 4. Optical coherence tomography of the graft for anterior lamellar keratoplasty after 1 day of preservation

The degree of swelling of turkey-derived xenografts used for ALK did not exceed that observed in human corneal grafts at any point during the observation period. As with human donor tissue, the extent of graft edema should be taken into account when preparing xenografts for clinical application.

After 5 days of hypothermic preservation in corneal storage solution, the mean thickness of the xenografts increased to 445 ± 61.5 μm. An example of graft evaluation using OCT after 5 days of storage is presented in Fig. 5, showing a central corneal thickness of 433 μm.

At the end of the 5-day preservation period, samples of the experimental corneal xenografts intended for an-

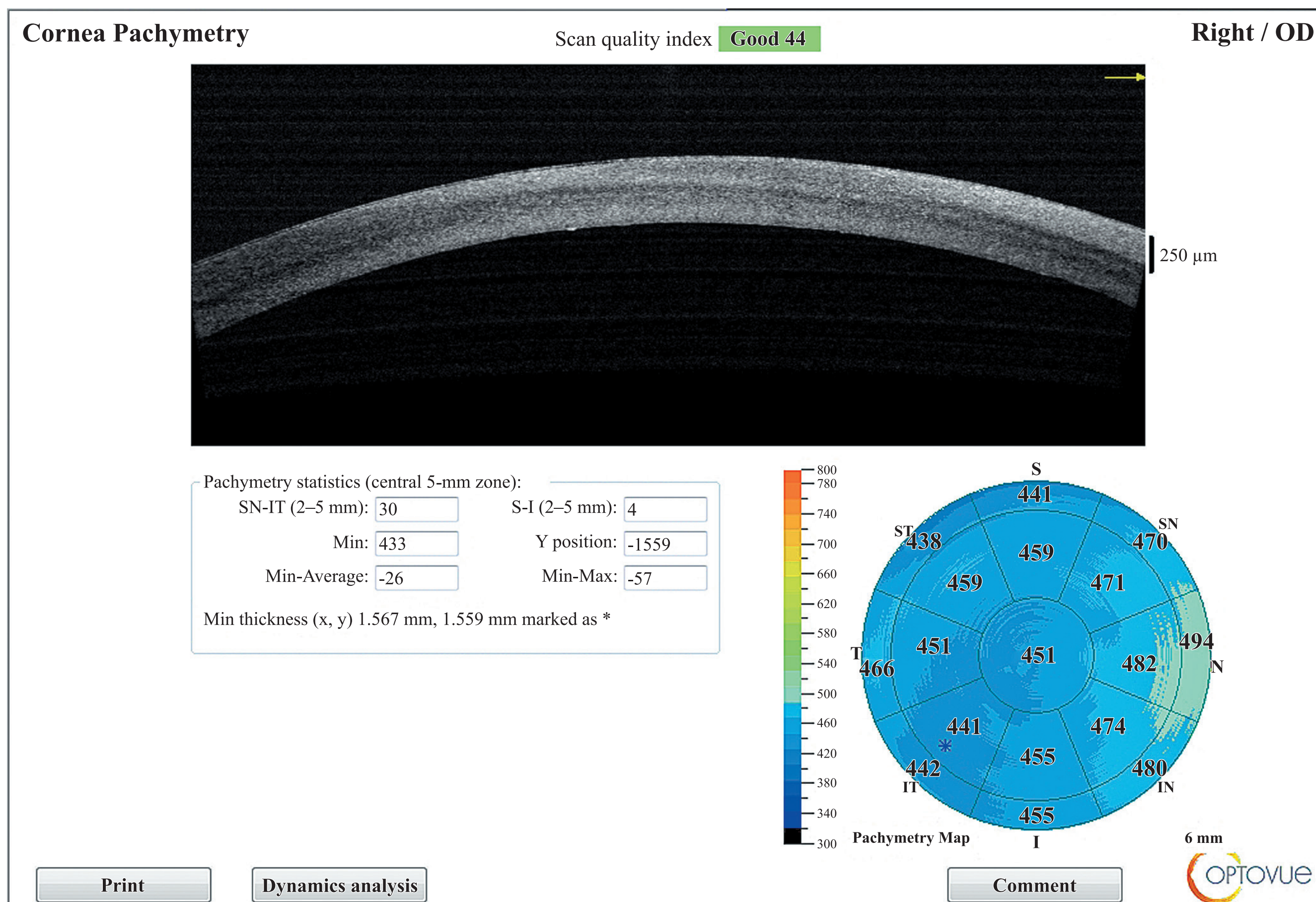

Fig. 5. Optical coherence tomography of the graft for anterior lamellar keratoplasty after 5 days of preservation

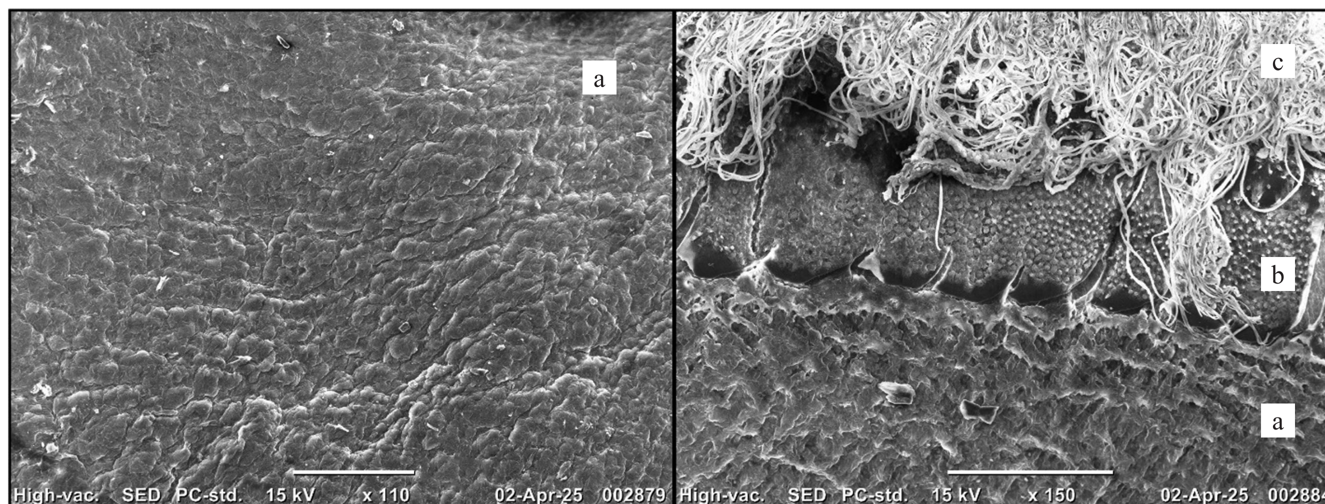

Fig. 6. Scanning electron microscopy of a turkey cornea after 5 days of hypothermic preservation: a, stromal surface after microkeratome treatment; b, endothelium at the periphery of the cornea; c, trabecular meshwork of the eye

terior lamellar xenokeratoplasty were examined using SEM. The analysis showed that the stromal surface, following microkeratome treatment, had a smooth topography, while the collagen fibers retained their characteristic cross-striated organization. Importantly, the endothelial layer composed of hexagonal cells remained preserved, indicating that turkey corneal tissue can withstand hypothermic storage for up to 5 days.

An example of a corneal image after 5 days of preservation, obtained by SEM, is presented in Fig. 6.

DISCUSSION

This study was aimed at the experimental evaluation of the structural characteristics of the turkey cornea and the assessment of its suitability as a xenogeneic material for selective keratoplasty, particularly ALK.

As indicated in the literature, investigations of cadaveric human donor corneas can be readily performed using standard ophthalmic diagnostic tools; however, such approaches may be more challenging when applied to xenogeneic tissues. In this context, the present study demonstrates, for the first time, the feasibility of performing confocal microscopy on native turkey eyes without prior enucleation, thereby enhancing the objectivity and reliability of the obtained data.

All measurements and image analyses were conducted by experienced specialists using calibrated instruments, ensuring consistency and reproducibility across the study.

As a result of this study, several key structural parameters of the turkey cornea were characterized for the first time using confocal microscopy. Mean corneal thickness was 508 ± 33.5 μm, with a stromal thickness of 440 ± 29.7 μm. Keratocyte density was 478 ± 43 cells/mm^2, and endothelial cell density was 4373 ± 532 cells/mm^2.

It was also shown that xenografts of the required thickness for ALK can be reliably prepared from turkey corneas using standard equipment at the Eye Tissue Bank, without the need for additional technical modifications. Furthermore, turkey cornea–derived xenografts were shown to tolerate hypothermic preservation in Borzenko–Moroz corneal storage medium for up to 5 days while maintaining structural integrity. This study presents some of the key parameters of the turkey cornea. At this stage, the findings suggest that the turkey cornea represents a promising alternative for xenokeratoplasty, since widely used porcine corneas differ substantially from human corneas in several critical characteristics, particularly thickness. For instance, the central thickness of the porcine cornea significantly exceeds that of the human cornea, which may necessitate modification or redesign of existing equipment used for preparing grafts for ALK.

In this regard, the turkey cornea shows a notable advantage over the porcine cornea, as its thickness is much closer to that of the human cornea. Given the critical role of the cornea in the optical system of the eye, such similarity is of particular importance. Unlike the porcine cornea, which exhibits relatively uniform thickness across the central and peripheral regions, both the turkey and human corneas have a thinner central zone with gradual thickening toward the periphery.

These structural differences limit the suitability of the porcine cornea as an optimal xenograft material for keratoplasty. For this reason, there is potential for using the turkey cornea in the development of a xenograft for keratoplasty as a new xenogeneic material.

CONCLUSION

The findings on the fundamental structural characteristics of the turkey cornea – including its overall morphology, corneal thickness, keratocyte density, endothelial cell density, nerve fiber distribution, and stromal collagen organization – support its characterization as a promising xenogeneic donor material for xenokeratoplasty. However, further study is required before it can be used in reconstructive corneal surgery.

The authors declare no conflict of interest.

REFERENCES

1. *Ackland P, Resnikoff S, Bourne R*. World blindness and visual impairment: despite many successes, the problem is growing. *Community Eye Health*. 2017; 30 (100): 71–73.
2. *Holland G, Pandit A, Sánchez-Abella L, Haiek A, Loinaz I, Dupin D et al*. Artificial Cornea: Past, Current, and Future Directions. *Front Med (Lausanne)*. 2021 Nov 12; 8: 770780. doi: 10.3389/fmed.2021.770780.
3. *Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, Thuret G*. Global Survey of Corneal Transplantation and Eye Banking. *JAMA Ophthalmol*. 2016 Feb; 134 (2): 167–173. doi: 10.1001/jamaophthalmol.2015.4776.
4. *Yoon CH, Choi HJ, Kim MK*. Corneal xenotransplantation: Where are we standing? *Prog Retin Eye Res*. 2021 Jan; 80: 100876. doi: 10.1016/j.preteyeres.2020.100876.
5. *Nascimento H, Martins TMM, Moreira R, Barbieri G, Pires P, Carvalho LN et al*. Current Scenario and Future Perspectives of Porcine Corneal Xenotransplantation. *Cornea*. 2025 Mar 1; 44 (3): 387–404. doi: 10.1097/ICO.0000000000003723.
6. *Kim MK, Hara H*. Current status of corneal xenotransplantation. *Int J Surg*. 2015 Nov; 23 (Pt B): 255–260. doi: 10.1016/j.ijsu.2015.07.685.
7. *Maghsoudlou P, Sood G, Gurnani B, Akhondi H*. Cornea Transplantation. 2024 Feb 24. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan. Available from: https://pubmed.ncbi.nlm.nih.gov/30969512/.
8. *Crawford AZ, Patel DV, McGhee CNj*. A brief history of corneal transplantation: From ancient to modern. *Oman*

J Ophthalmol. 2013 Sep; 6 (Suppl 1): S12–S17. doi: 10.4103/0974-620X.122289.

9. *Hara H, Cooper DK*. Xenotransplantation – the future of corneal transplantation? *Cornea*. 2011 Apr; 30 (4): 371–378. doi: 10.1097/ICO.0b013e3181f237ef.
10. *Song YW, Pan ZQ*. Reducing porcine corneal graft rejection, with an emphasis on porcine endogenous retrovirus transmission safety: a review. *Int J Ophthalmol*. 2019 Feb 18; 12 (2): 324–332. doi: 10.18240/ijo.2019.02.21.
11. *Islam R, Islam MM, Nilsson PH, Mohlin C, Hagen KT, Paschalis EI et al*. Combined blockade of complement C5 and TLR co-receptor CD14 synergistically inhibits pig-to-human corneal xenograft induced innate inflammatory responses. *Acta Biomater*. 2021 Jun; 127: 169–179. doi: 10.1016/j.actbio.2021.03.047.
12. *Shi Y, Bikkuzin T, Song Z, Jin X, Jin H, Li X, Zhang H*. Comprehensive evaluation of decellularized porcine corneal after clinical transplantation. *Xenotransplantation*. 2017 Nov; 24 (6): e12338. doi: 10.1111/xen.12338.
13. *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 Jul; 26 (4): e12509. doi: 10.1111/xen.12509.
14. *Zheng Q, Zhang Y, Ren Y, Zhao Z, Hua S, Li J et al*. Deep anterior lamellar keratoplasty with cross-linked acellular porcine corneal stroma to manage fungal keratitis. *Xenotransplantation*. 2021 Mar; 28 (2): e12655. doi: 10.1111/xen.12655.
15. *Liu XN, Zhu XP, Wu J, Wu ZJ, Yin Y, Xiao XH et al*. Acellular ostrich corneal stroma used as scaffold for construction of tissue-engineered cornea. *Int J Ophthalmol*. 2016 Mar 18; 9 (3): 325–331. doi: 10.18240/ijo.2016.03.01.
16. *Nesterov AP*. Experimental studies on transplantation of heterogeneous cornea: Abstract of Cand. Sci. (Medicine) dissertation. Kuibyshev, 1956; 20.

The article was submitted to the journal on 8.07.2025