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Current Progress in Corneal Xenotransplantation

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Abstract

Blindness is a devastating situation, and one of the common causes is corneal blindness. Corneal transplantation is the standard treatment for the corneal blindness. The lack of human donors demands the exploration of alternative treatments such as corneal xenotransplantation and bioengineered corneas. We review the researches regarding immunological and physio-anatomical barriers of corneal xenotransplantation, recent progress of corneal xenotransplantation in nonhuman primate studies, and updates of regulatory guidelines to conduct clinical trials for corneal xenotransplantation. The current development of genetically-engineered and gene-editing technologies suggests that the promise much for the field of xenotransplantation. A clinical trial of xenotransplantation using a cellular porcine corneal stroma has already been conducted; however, safety concerns have not been reported so far. With regard to the regulatory aspects and preclinical efficacies, corneal xenotransplantation has become one of the clinically realistic options as human substitutes and progress in recent research is promising to advance corneal xenotransplantation field.

Keywords: cornea, clinical trial, nonhuman primate, regulatory guidelines, transplantation, xenotransplantation

1. Introduction

Blindness is a devastating situation with an estimated 39 million cases worldwide, and one of the common causes is corneal blindness [1]. Corneal transplantation is the standard treatment for the corneal blindness. According to “Cost-benefit analysis of corneal transplant,” which had been reported by Eye Bank Association of America and the Lewin group in 2013, the net lifetime benefit from the transplantation was estimated at \$118,000, whereas the medical cost of the transplant was \$16,500 [2]. However, supply of the donor cornea cannot meet the demand in developing countries, and in near future, the number of the eligible cornea

will be reduced in the aged societies of the developed countries [1, 3, 4]. Another reason to seek a substitute for allograft is that ethical concerns about organ trafficking [2, 5]. The lack of human donors and the ethical concerns regarding the human organ trafficking drive the need to explore alternative treatments such as corneal xenotransplantation and bioengineered corneas [2, 6–12]. When a survey was conducted through a telephonic interview to assess how corneal xenotransplantation will be perceived by the society, 42.4% of the individuals in the wait-list for corneal allotransplantation expressed favorable views on corneal xenotransplantation [13].

Cornea is considered applicable as a xenograft, because the eye is regarded as an immune-privileged site. Surprisingly, Dr. Kissam was the first one who conducted pig-to-human corneal xenotransplantation in 1844, although the pig cornea did not survive [14]. Current progress in genetically engineered (GE) pigs and development in gene editing made by clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology have made xenotransplantation a possible option for human application [15–21]. Recent advances in corneal xenotransplantation through the success in primate studies and the establishment of international regulatory guidelines have brought us a step closer to apply xenograft in clinical trials [22–25]. In fact, clinical trial of lamellar corneal transplantation using a decellularized porcine graft had been already conducted in human subjects in China to treat fungal ulcers [26].

This chapter reviews the current knowledge of immunological and physiological barriers of corneal xenotransplantation, recent progress of corneal xenotransplantation in animal studies, and updates of regulatory guidelines in order to conduct clinical trials of corneal xenotransplantation.

2. Anatomy and physiology in corneal transplantation

A cornea is an avascular and transparent collagenous tissue with a critical role in vision by transmitting and refracting a light in order to focus the light on the macula. Adult human cornea measures 11–12 mm horizontally and 9–11 mm vertically [27]. It is approximately 500–550 μm thick in the center and 700 μm thick in the periphery [27]. The refractive power of the cornea is 40–44 diopters [27].

The cornea consists of three different cellular layers and two interfaces; the epithelial cell layer, Bowman's layer (interface), the stroma containing keratocytes (fibroblasts), Descemet's membrane (interface), and the endothelial cell layer (**Figure 1**) [27]. The thickness of the corneal epithelial layer is approximately 50 μm . Stem cells of the epithelium reside in the limbus, which is located in the peripheral junction between the cornea and the conjunctiva [27]. The stroma constitutes the largest portion, accounting for more than 90% of the total corneal thickness [27]. The uniform arrangement and continuous slow turn-over of the collagen fibers by keratocytes are essential for corneal transparency [27]. A single layer of corneal endothelial cells covers the posterior surface of Descemet's membrane, and it keeps the cornea transparent by actively pumping out the water from the stroma using Na^+ - and K^+ -dependent ATPase against imbibition pressure [27].

Figure 1. Normal anatomy of the cornea and schematic figures of the different types of the keratoplasties. (A) Normal histology of a rabbit cornea in hematoxylin and eosin staining. (B) Schematic figure of a normal cornea which consists of three different cellular layers and two interfaces. (C) Penetrating keratoplasty (PK); a procedure of full thickness replacement of the cornea. (D) Anterior lamellar keratoplasty (ALK); a procedure of partial thickness replacement of the anterior cornea. (E) Deep anterior lamellar keratoplasty (DALK); a procedure of almost the full thickness of stromal layers except Descemet's membrane. (F) Endothelial keratoplasty (EK); a procedure of replacement of the corneal endothelium including Descemet's membrane or posterior stroma.

The cornea is one of the few tissues in the body that enjoy immune-privileged status by passively ignoring or actively modulating immunological reactions [28, 29]. Normal and healthy cornea is devoid of vessels and lymphatic channels, thereby shielding it from immune-mediated attacks by preventing transport of antigens and antigen-presenting cells and thus attenuating the access of immune cells to the graft [28, 29]. Weak or absence of expression of major histocompatibility complex (MHC) class I and II antigens on the corneal cells is also related to the immune privilege of the cornea [29]. In addition, the cornea expresses various cell membrane-bound or soluble immunomodulatory molecules such as Fas ligand (FasL, CD95L), complement regulatory proteins (CRPs), tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), programmed death-ligand 1 (PD-L1), and MHC-Ib that are capable of suppressing immune cells [29]. Interestingly, eye has a unique immune suppression mechanism called anterior chamber-associated immune deviation (ACAID) [29]. In corneal transplantation, the donor allografts are directly contacted with the AC to induce ACAID, a distinctive systemic immune response to alloantigen [28]. ACAID is an active process that induces antigen-specific CD4⁺ and CD8⁺ T regulatory cells (Tregs) capable of suppressing cellular immune

responses and protecting a graft from immune rejection in transplantation [29]. However, any infectious or inflammatory events may break down the immunological privilege of the cornea.

The history of corneal transplantation using allografts and xenografts dates back to more than two centuries [3]. Penetrating keratoplasty (PK), a procedure of full thickness replacement of the cornea, has been used as the dominant procedure worldwide [3]. It is a successful method for most causes of corneal blindness. Lamellar transplantation surgery, that selectively replaces only diseased layers of the cornea, consists of anterior lamellar keratoplasty (ALK) and deep anterior lamellar keratoplasty (DALK) [3]. ALK usually replaces partial thickness of the anterior stromal layers and it may induce interface haze between the graft and the recipient corneal stroma. DALK replaces almost the full thickness of stromal layers except Descemet's membrane and endothelial cell layer without inducing interface haze. Both procedures can be applied to patients who have a corneal opacity with an intact endothelial cell layer, and they can eliminate the risk of endothelial rejection [3]. Endothelial keratoplasty (EK) can selectively replace the corneal endothelium in patients with endothelial disease. Rejection risk in PK is higher rather than that in ALK/DLAK or EK [3]. Different types of keratoplasties are schematically shown in **Figure 1**.

3. Immunological barriers of corneal xenotransplantation

Although an eye is an immune-privileged site, the innate, humoral, and cellular immune responses are involved in corneal allograft rejection. These immune reactions also happen in corneal xenograft rejection associated with pig antigens. Galactose- α -1,3-galactose (e.g. α Gal) to which human natural Ig M antibodies are reactive is constantly expressed on porcine cells. This is a critical obstacle to overcome hyperacute xenogeneic rejection in most organ transplantation [30]. Therefore, the distribution of porcine antigens (e.g., α Gal, non-Gal) in the cornea has been investigated. It has been found that wild type (WT) porcine cornea expresses α Gal mostly in the anterior stromal keratocytes in immunohistochemical or immunofluorescent staining [31, 32]. *In vitro* culture, α Gal expression appears on both WT porcine endothelial cells and keratocytes [32]. Based on mass spectrometry, sialylated N-glycans have been identified from both WT porcine corneal endothelial cells and keratocytes [33]. As non-Gal antigens, N-glycolylneuraminic acid (NeuGc) as well as N-acetyl sialic acid (NeuAc) are also identified in both WT corneal endothelial cells and keratocytes [33]. Since α 1,3-galactosyl-transferase gene-knockout (GTKO) pigs that do not express the Gal epitopes have been made [15, 34], the feasibility of GTKO pigs is investigated for the corneal xenograft. In immunofluorescent staining, strong expression of NeuGc has been found in all layers of both WT and GTKO pig corneas [35]. That is to say, both α Gal and non-Gal epitopes are widely expressed in WT cornea, whereas antigenic epitopes such as non-Gal are still expressed in GTKO cornea.

In vitro study has shown that IgG antibody binding affinities to the cornea or the T cell responses of GTKO pigs are weaker than those of WT pig corneas [35, 36]. NeuGc is a major target of human antibodies, but not a target of nonhuman primate (NHP) antibodies [37, 38]. The absence of α Gal or NeuGc on porcine peripheral blood mononuclear cells or corneal cells can significantly

decrease human antibody binding significantly *in vitro* [39, 40]. However, when immune reactions are compared between GTKO/hCD46 and GTKO/hCD46/NeuGc KO pigs, the strength of the human T-cell proliferative response to GTKO/hCD46/NeuGc KO pig cells is similar to that to GTKO/hCD46 pig cells. The absence of NeuGc expression on GTKO/hCD46 pig cells does not diminish human platelet aggregation or decreases the instant blood-mediated inflammatory reaction (IBMIR) to pig cells [41]. In an NHP study, GT KO/CD46 pig corneas are not associated with prolongation of the graft survival or a reduced antibody response compared with WT pig corneas [42]. Taken together, it remains doubtful whether the absence of α Gal or NeuGc expression on cornea of the GE pigs might have an advantage over WT cornea in *in vivo* xenotransplantation.

Major histocompatibility complex (MHC) antigens play important roles in corneal allotransplantation [43–45]. Therefore, MHC antigens might have roles in corneal xenotransplantation as in other organ xenotransplantations [46, 47]. In fact, human antiporcine T cell response and binding property of IgG HLA-specific antibodies to pig lymphocytes are similar to an allogeneic responses with both direct and indirect pathways of recognition in the human antiporcine MHC class II responses being functionally intact [48–50]. In DNA microarray, MHC-A has been expressed in both WT porcine corneal keratocytes and endothelial cells [51]. Genetically-engineered Class I MHC knockout pigs have reduced levels of CD4⁺CD8⁺ T cells in peripheral blood [52]. Modulation of swine MHC by transferring human HLA DPw0401 can reduce human-to-pig cellular response, *in vitro* [53]. Human dominant-negative class II transactivator (CIITA-DN) transgenic pigs that can suppress swine leukocyte antigen (SLA) class II expression have been found to have reduced human T cell response, *in vitro* [54]. Although MHC-related immune response is evidently important in xenotransplantation, *in vitro* and *in vivo* immune responses against porcine MHCs in corneal xenotransplantation have not been published yet.

An unmodified cellular porcine cornea is defined as a xenotransplant medicinal product, while a decellularized porcine cornea is defined as a medical device [25]. As a medical device, porcine decellularized cornea can be produced in various ways to reduce immunogenicity [55–58]. Decellularized porcine cornea has an advantage on the survival of the graft by reducing immune responses in different animal models as well as in human clinical study [23, 26, 56, 57, 59, 60].

4. Rejection mechanism in corneal xenotransplantation through various *in vivo* animal models

In corneal allotransplantation, a CD4⁺ T cell-mediated reaction is primarily involved in graft rejection [8, 61–63], while CD8⁺ T cell- and complement-mediated reactions are partially involved in allograft rejection [64–67].

Rejection mechanisms of corneal xenotransplantation have been investigated using various animal models (Table 1) [8, 23, 24, 42, 68–76]. The main rejection mechanism seems to be different depending on the animal model used. Unlike xenotransplantation of the vascular organs, hyperacute rejection (minutes to hours) is not presented in all corneal xenotransplantation models [4, 8].

Models	Median survival (days)	Proposed rejection mechanism
Lewis rat-to-guinea pig [68]	8	IgM and IgG xenoantibody
Guinea pig-to-rat [69]	7	T cell, neutrophil, macrophage, Ig G
Guinea pig-to-mouse [70, 71]	9–16	CD4 ⁺ T cell, complement, MHC class II
Lewis rat-to-mouse [72]	9.4	CD4 ⁺ T cell, antibody
Pig-to-mouse [73, 74]	9.0–9.4	CD4 ⁺ T cell, macrophage, complement
Pig-to-GTKO mouse [75]	9.0	IgG α Gal antibody, CD4 ⁺ and CD8 ⁺ T cell, macrophage
WT pig-to-NHP [23, 24]	26*–189.8**	CD4 ⁺ or CD8 ⁺ T cell, macrophage, B cell, IgG/IgM antibody, complement
GTKO/CD46 pig-to- NHP [42]	104	CD3 ⁺ T cell, non-Gal pig antibody
hCTLA4-Ig pig-to-NHP [76]	70.3	Macrophage, CD3 ⁺ CD4 ⁺ T cell, CD79 ⁺ B cell

GTKO, α 1,3-galactosyltransferase gene-knockout; WT, wild type; NHP, nonhuman primate; CD46, membrane cofactor protein (MCP).

*Survival of full-thickness keratoplasty (PKP).

**Survival of anterior lamellar keratoplasty (ALK).

Table 1. Rejection mechanisms of the corneal xenotransplantation in various animal models.

In Lewis rat-to-guinea pig corneal transplantation, the mean survival time of corneal xenografts has been reported to be 8 days with IgM and IgG xenoantibody production after pre-sensitization [68]. In Guinea pig-to-rat model, the mean survival time of corneal xenografts is reported to be 7 days with a IgG deposition and infiltration of T cells, neutrophils, and macrophages in the graft [69]. In guinea pig-to-mouse corneal xenotransplantation, the median survival time is 9–16 days in wild types, whereas the survival time is extended in mice deficient in the CD4, C3, or MHC class II gene, suggesting that CD4⁺ T cells, complement, and host antigen-presenting cells might contribute to graft rejection [70, 71]. In Lewis rat-to-mice corneal xenotransplantation, survival time (mean survival time of 44.1 days) of xenograft is found to be longer after treatment with antiCD4 antibody compared to that of the control (mean survival of 9.4 days). However, xenografts treated with antiCD4 antibody are rapidly rejected by antibody-containing serum (mean survival of 21.5 days) [72]. In pig-to-mouse corneal xenotransplantation, median survival time is 9.0 days with macrophages and CD4⁺ T cells being found in rejected grafts in WT mice, and the survival time is extended in severe combined immunodeficiency (SCID) mice [73]. Natural killer (NK) cells are not involved in the xenogeneic rejection in this model [73]. In pig-to-mouse corneal xenotransplantation, complement depletion has prolonged the survival of xenograft, showing deposition of IgG and IgM in rejected grafts [74]. In pig-to-GTKO mouse corneal xenotransplantation, gradual increase of IgG α Gal antibody is evident suggesting that α Gal might affect the long-term survival of pig corneal xenografts through antibody-mediated reactions [75].

In pig-to-nonhuman primate (NHP) corneal xenotransplantation, grafts are not hyperacutely rejected, regardless of pig genotypes [7]. In WT pig-to-NHP corneal xenotransplantation,

infiltrations of CD4⁺ or CD8⁺ T cells, macrophages, and B cells and deposits IgG/IgM and C3c have been observed in rejected grafts [23, 24]. It indicates that both the cellular and humoral responses are involved in WT corneal xenograft rejection of NHP models as in allograft rejection. In GT KO/CD46 (human complementary regulatory protein) pig-to-NHP corneal xenotransplantation, CD3⁺ T lymphocytes still infiltrate in the graft accompanied by increased non-Gal pig antibodies in the blood [42]. Cell infiltration in rejected hCTLA4Ig transgenic grafts is mainly composed of macrophages with CD3⁺, CD4⁺ T, and CD79⁺ B cells to a lesser extent than those in WT types of grafts [76]. It indicates that T cell- and antibody-mediated reactions cannot be exempted even in GE pig grafts.

5. Anatomical barriers in corneal xenotransplantation

To restore a vision in corneal xenotransplantation as a functional success, anatomical (e.g., diameter, thickness, and tensile strength), physiological (e.g., cellular behaviors), and optical (e.g., refractive power for light to focus on the retina) properties of the substitute cornea should be similar to those of a human cornea. In this regard, WT or GTKO pig cornea is considered as a potential alternative to human cornea (**Table 2**) [4, 7, 77–86].

A major anatomical barrier in corneal xenotransplantation is the difference in corneal thickness between the human recipient and the pig donor. Pig corneal thickness and endothelial cell density are dependent on the age and the breed as shown in **Table 2** [7, 77–79, 81–83]. Pig central corneas are thicker (659–995 μm) than human central corneas (average; 536 μm). The donor thickness should be in the range so that peripheral edges of the cornea between donor and recipient can be appropriately approximated. Unlike human cornea with center to peripheral thickness difference by 150–250 μm , there is no significant difference in the thickness between central (666 μm) and peripheral locations (657–714 μm) of pig cornea [81]. Consequently, a pig cornea whose central thickness is thicker than in human is considered applicable in human in surgical aspect. However, no paper has documented that pig corneal graft with a central thickness of more than 950–1000 μm is capable of being transplanted up to date. Tensile strength of the pig cornea is similar to that of the human cornea which is operable for corneal transplantation, although stress-relaxation of the pig cornea is significantly lower than that of the human cornea [4, 84]. Differences in stress-relaxation do not affect the long-term mechanical maintenance of the graft in NHP studies. Optical power of the pig cornea has been found to be comparable to that of the human cornea [82, 83, 85].

The cornea can maintain transparency by functionally intact corneal endothelial cells. Therefore, endothelial density and proliferative potential in the endothelial cells of the pig cornea should be similar to those of human cornea. The proliferative potentials of pig and human endothelial cells are similar to each other [77, 79]. Endothelial cell density of the pig cornea is decreased depending on age, as similar to that of aged human [77–79, 86]. However, the age-dependent decrease of endothelial cell density in GE pigs ($1714.0 \pm 19.2 \text{ mm}^{-2}$ in 20–25 months old) is higher than that in WT pigs ($2130.2 \pm 193.7 \text{ mm}^{-2}$ in 42 months old) [78]. Considering that more than 2200 mm^{-2} of the endothelial cell density is preferred for

Parameters and breed of the pig	Pig	Human	Mean pig age (months)
Central corneal thickness (μm)			
GE pig (Revivicor, Blacksburg, VA)	659 [78]	536 [80]	1.5
WT Danish Landrace pig (Lars Jonsson Lynge, Denmark)	666 [81]		3.5
WT pig (Wally Whippo, Enon Valley, PA)	775 [78]		5–10
WT SNU miniature pig (Seoul, Korea)	833 [77]		42
Yorkshire pig (Seoul, Korea)	867 [82]		4
GE pig (Revivicor, Blacksburg, VA)	868 [78]		15
Sus scrofa domestica	877 [83]		6–8
GE pig (Revivicor, Blacksburg, VA)	914 [78]		20–25
WT pig (Wally Whippo, Enon Valley, PA)	995 [78]		42
Tensile strength (MPa) [84]	3.70	3.81	NA
Stress-relaxation pattern [*] ; <i>P</i> (×100) [84]	64.6 ^a	85.6	NA
Stress-relaxation pattern [*] ; <i>K</i> (–) [84]	0.0553 ^a	0.0165	NA
Corneal power (Diopter)	40.2 [82, 83]	43.7 [85]	4–8
Endothelial cell density (/mm²)			
WT pig (Wally Whippo, Enon Valley, PA)	3094 [78]	2720 [86]	5–10
GE pig (Revivicor, Blacksburg, VA)	3022 [78]		15
WT SNU miniature pig (Seoul, Korea)	2625 [77]		42
WT pig (Wally Whippo, Enon Valley, PA)	2130 [78]		42
GE pig (Revivicor, Blacksburg, VA)	1714 [78]		20–25
The data present average of the parameters.			
WT, Wild-type; GE; genetically engineered; NA, not available data.			
[*] <i>P</i> is the value of <i>G</i> (<i>t</i>) at the end of the stress-relaxation test; <i>K</i> is the slope of fitted <i>G</i> (<i>t</i>)-ln <i>t</i> line.			
^a <i>p</i> < 0.01 compared with Stress-relaxation pattern in human.			

Table 2. Anatomical, physiological, and optical properties of the pig cornea compared to those of adult human cornea.

a donation, the age of the pig as a donor should be limited in accordance with endothelial cell density. The age limitation of GE pigs might be different from that of WT pigs. Unlike type-dependent differences of endothelial cell density (WT versus GE), the preservation time-dependent decrease of endothelial cell density in WT pig cornea is not different from that in human cornea [77]. The preservation time-dependent decrease of endothelial cell density in GE pig cornea is not reported.

6. Efficacy of corneal xenotransplantation and current progress in *in vivo* animal studies

Survival of a corneal allograft or xenograft is affected by immunologic reaction, graft size, the presence of corneal endothelial cells, and the hierarchical discordancy between the donor and the recipient [87–92]. Therefore, we should compare the survival time of xenografts depending on the various animal models in consideration with the aforementioned risk factors.

Reported results on the survival time of different types of the pig grafts in various animal models are shown in **Tables 3** and **4**. Outcome for small and medium sized animal models is shown in **Table 3**. Decellularized graft survives longer than fresh grafts, and anterior lamellar partial thickness graft without including the endothelial cell layer survives longer than posterior lamellar or full thickness graft that includes the endothelial cell layer (**Table 3**) [56, 57, 60, 73, 93–95].

Type of pig donor	Recipient	Graft size (mm)	Graft thickness	Median survival (days)
Fresh	C57BL/6 mice	3.0	Posterior lamellae*	9.0 [73]
Fresh	BALB/C mice	3.0	Posterior lamellae*	9.0 [73]
Fresh	Sprague-Dawley rats	6.0	Posterior lamellae*	9.3 [93]
Fresh	Sprague Dawley rats	2.0	Anterior lamellae	14.0 [94]
Decellularized ^c	Sprague Dawley rats	2.0	Anterior lamellae	28.0 [94]
Fresh	Rabbits	7.0	Anterior lamellae	29.1 [95]
Fresh	Rabbits	7.0	Full thickness	16.8 [95]
Decellularized*	Rabbits	8.0	Anterior lamellae	>180 [57]
Decellularized ^{§§}	Rabbits	6.3	Anterior lamellae	84 [60]
Decellularized**	Rabbits	10.0	Anterior lamellae	365 [56]

*Posterior lamellae that includes endothelial cell layer (Anterior lamellae does not include endothelial cell layer).

^cLyophilized graft.

^{*}Treated with hypertonic saline.

^{§§}Treated with 200 U/ml phospholipase A2 and 0.5% sodium deoxycholate.

^{**}Treated with sodium dodecyl sulfate.

Table 3. The median survival time of various types of the pig grafts in small- or medium-sized animal models.

Type	Donor pig	Recipient (number)	Immunosuppression	Survival (days)	Reported year
ALK	WT	Cynomolgus (<i>n</i> = 6)	None	>30*, >30*, >30*, 75, 165, 180	2003 [31]
ALK	WT	Rhesus (<i>n</i> = 4)	None	>90, >90, >90, >90	2007 [22]
ALK	WT	Rhesus (<i>n</i> = 5)	None	180, 15, 180, 180, 180	2011 [97]
ALK	WT	Rhesus (<i>n</i> = 4)	Local and systemic steroid	>398, >194, 24.5, 24.5	2011 [23]
ALK	WT	<i>Macaca fascicularis</i> (<i>n</i> = 4)	Local steroid	9, 70, 21, 21	2014 [76]
DALK	WT	Rhesus (<i>n</i> = 5)	Steroid+antiCD40 antibody	>389, >382, >236, >201, >61	2017 [99]
ALK*	WT	Rhesus (<i>n</i> = 5)	None	180, 180, 180, 180, 180	2011 [97]
ALK*	WT	Rhesus (<i>n</i> = 5)	Local steroid	180, 180, 180, 180, 180	2011 [97]
ALK*	WT	Rhesus (<i>n</i> = 5)	Local and systemic steroid	>391, >265, >208, >195, 28	2011 [23]
ALK	hCTLA4-Ig transgenic	<i>Macaca fascicularis</i> (<i>n</i> = 4)	Local steroid	21, 50, 90, 120	2014 [76]
ALK	GTKO/hCD39/hCD55/hCD59/FT	<i>Macaca fascicularis</i> (<i>n</i> = 2)	Local steroid	9,34	2014 [76]
PKP	WT	Rhesus (<i>n</i> = 4)	Local steroid	129, 276, 182, 144	2007 [22]
PKP	WT	Rhesus (<i>n</i> = 6)	Cyclophosphamide+BMT	32, 42, 40, 34, 38, 30	2013 [98]
PKP	WT	Rhesus (<i>n</i> = 6)	Cyclophosphamide	12, 18, 16, 20, 20, 20	2013 [98]
PKP	WT	Rhesus (<i>n</i> = 3)	Local and systemic steroid	21, 28, 29	2015 [24]
PKP	WT	Rhesus (<i>n</i> = 4)	Local and systemic steroid + antiCD154 antibody	>933, >243, 318, >192	2015 [24]
PKP	WT	Rhesus (<i>n</i> = 4)	Local steroid	157, 28, 92, 33	2017 [42]
PKP	GTKO/CD46	Rhesus (<i>n</i> = 4)	Local steroid	128, 57, 47, 171	2017 [42]

ALK, anterior lamellar keratoplasty (partial thickness); BMT, bone marrow transplantation; DALK, deep anterior lamellar keratoplasty; FT, fucosyl transferase; GTKO, α 1,3-galactosyltransferase gene-knockout; hCTLA4-Ig, human cytotoxic T-lymphocyte-associated antigen4-immunoglobulin; hCD39, human ectonucleoside triphosphate diphosphohydrolase-1; CD46, membrane cofactor protein (MCP); hCD55, human complement decay-accelerating factor; hCD59, human MAC-inhibitory protein; PKP, penetrating keratoplasty (full thickness).

*Sacrificed at 1 month for histology.

*Decellularized cornea.

Table 4. Current progress on clinical efficacies in pig-to-NHP corneal xenotransplantation from 2003 to 2017.

Current progress on clinical efficacies in pig-to-NHP corneal xenotransplantation from 2003 to 2017 is shown in **Table 4** [7, 22–24, 31, 42, 76, 96–99]. Some studies have presented encouraging outcomes in lamellar or full-thickness corneal xenotransplantation with or without immunosuppressants. The survival time varies depending on the breed of the donor and recipients, immunosuppressive protocols, and types of the corneal grafts. Processed acellular corneas can prolong the survival time of ALK. With steroid treatment, partial thickness corneal transplantation that does not include endothelial cell layer (ALK) shows better survival than full thickness corneal transplantation (PKP). GE pigs in ALK or PKP do not show significant increase of the survival time compared to the control. With antiCD154 treatment, PKP using WT Seoul National University (SNU) miniature pig has demonstrated the longest survival time in the NHP model. Taken together, corneal xenotransplantation using fresh pig graft still requires stronger immunosuppressant than steroid alone, regardless of the type of donor pig (WT or GE).

7. Updates on regulatory aspects of corneal xenotransplantation

In 2013, the first consensus on guidelines for clinical trials of corneal xenotransplantation has been established in Korea [87]. Thereafter, international consensus statement on conditions for undertaking clinical trials of xenocorneal transplantation has been finally published in International Xenotransplantation Society (IXA) in 2014 [25]. IXA consensus statements on conditions for clinical trials of corneal xenotransplantation include the followings; (1) ethical requirement, (2) quality control of source pigs, (3) quality control of pig corneal products, (4) preclinical efficacy and safety data that are required to justify a clinical trial, (5) strategies to prevent porcine endogenous virus transmission (PERV) transmission, and (6) patient selection and informed consent.

Key ethical requirements for clinical trials of corneal xenotransplantation are essentially identical to those required in other areas of clinical trials. These guidelines adhere to the basic ethical principles for clinical trials of islet xenotransplantation established by the Ethics Committee of the IXA and the Changsha Communique of the World Health Organization [25, 100]. Regulatory guidelines for pig sources and strategies to prevent porcine endogenous virus transmission (PERV) are basically the same as those for clinical trials of islet xenotransplantation [101–103].

Guidelines for corneal-specific issues have been intensively discussed on the procurement of porcine corneal products, preclinical efficacy, and safety data to justify initiation of a clinical trial, and inclusion criteria of the subjects. In order to be enrolled, the subject must meet the following criteria; (1) must be diagnosed with legal blindness as defined by the American Medical Association and the United States Congress as best corrected visual acuity of 20/200 or less in the better eye, (2) must be diagnosed with a corneal blindness that can be only cured with a corneal transplantation, (3) must not have timely access to receive corneal allotransplantation, (4) must be over the legal age, (5) must not be pregnant, must not plan to become pregnant, and must not be breast feeding, and (6) should be highly compliant. Keratoconus should be excluded due to the excellent allograft survival and younger age of the subject. Guideline for visual acuity can be exempted in a subject who requires an emergency

operation for actual or impending corneal perforation. Regarding adequate procurement of the corneal xeno-product, the guidelines of the European Eye Bank Association (EEBA) on the preparation of human corneal tissue should be adopted under provision that laboratory tests have confirmed that biological properties of the preserved pig cornea based on EEBA guidelines are comparable to those of the preserved human cornea. To prove preclinical efficacy, NHP data that the pig cornea xenograft should survive for more than 6 months in five of eight consecutive NHPs are required (ideally for 12 months in one or two successful cases). Compared to the 5-year survival rate (70–80%) of the islet allotransplantation, mean 5-year survival rate of corneal allotransplantation among the various corneal diseases is similar to each other (70–80%) [104–106]. Therefore, the same preclinical efficacy that has been accepted for islet xenotransplantation can be applied to corneal xenotransplantation with provisional condition that patient who is diagnosed as keratoconus must be excluded.

In 2016, the IXA consensus statement on conditions for undertaking clinical trials of porcine islet products has been revised for the first time [107–114]. New or under-appreciated topics have been discussed and updated regarding regulatory framework, genetic modification of the source pig, recipient monitoring for preventing disease transmission, patient selection, porcine islet product manufacturing, and quality control of source pigs. To undertake clinical trials of corneal xenotransplantation, under-appreciated topics as follows should also be addressed and revised [2]. (1) In source pigs, PERV-C negative donor pigs should be considered preferable, and donor pig selection criteria should be primarily based on low PERV expression levels and the lack of infectivity. (2) Clinical trial protocols using GE pig products also need to be assessed on a case-by-case basis. (3) For preclinical efficacy in corneal xenotransplantation, the finding that survival in four of six (or five of eight) consecutive NHP experiments may be sufficient to indicate potential success of a clinical trial that is similar to those in islet xenotransplantation. (4) Clinically relevant microorganisms should be included in pig screening programs. (5) When microorganisms are confirmed to be absent in the donor pig by sensitive microbiological examination, recipients need not to be monitored. (6) Life-long surveillance for PERV should be adjusted based on the clinical sign and the laboratory test if the subjects do not show any suspicious sign of PERV infection by sensitive laboratory examination for 2 years. In a clinical trial of islet cell xenotransplantation using microencapsulated pig islets, PERV DNA and PERV RNA are not detected in peripheral blood up to 113 weeks by real-time RT-PCR [115]. In this clinical trial, the subjects were followed-up for two years. If the risk of PERV transmission is proved to be negligent, follow-up time should be adjusted accordingly. Given that substantial scientific progress has been made in islet xenotransplantation and cornea field, the international consensus statement on corneal xenotransplantation is expected to be updated regarding these under-appreciated issues.

8. Future perspectives

Due to progresses made in immunosuppressive protocols, the availability of GE pigs, and appropriate guidelines for clinical trials, corneal xenotransplantation using pig cornea might be a feasible option to solve the shortage of donor corneas in the future. Decellularized porcine

graft also appears to be efficient in a clinical trial. Results of recent experiments of the corneal xenotransplantation in NHP models using cellularized pig grafts are encouraging, and it helps us decide whether we should keep developing xeno-related products of cornea. With better understanding on the antigenicity of pig cornea and the rejection mechanism involved in corneal xenotransplantation, optimized and standardized immunosuppression should be established before conducting a human clinical trial. As for fresh corneal grafts from GE pigs, the further experiments need to be performed to verify their efficacies as substitutes for human corneas.

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References

- [1] Lamm V, Hara H, Mammen A, Dhaliwal D, Cooper DK. Corneal blindness and xenotransplantation. *Xenotransplantation*. 2014;**21**(2):99-114. DOI: 10.1111/xen.12082
- [2] Kim MK. Commentary: Current status and future of corneal xenotransplantation. *Transplantation Technologies & Research*. 2016;**6**:3
- [3] Tan DT, Dart JK, Holland EJ, Kinoshita S. Corneal transplantation. *Lancet*. 2012;**379**(9827):1749-1761. DOI: 10.1016/s0140-6736(12)60437-1
- [4] Hara H, Cooper DK. Xenotransplantation—The future of corneal transplantation? *Cornea*. 2011;**30**(4):371-378. DOI: 10.1097/ICO.0b013e3181f237ef
- [5] Treasure T. The Falun Gong, organ transplantation, the holocaust and ourselves. *Journal of the Royal Society of Medicine*. 2007;**100**(3):119-121. DOI: 10.1258/jrsm.100.3.119
- [6] Ekser B, Ezzelarab M, Hara H, van der Windt DJ, Wijkstrom M, Bottino R, et al. Clinical xenotransplantation: The next medical revolution? *Lancet*. 2012;**379**(9816):672-683. DOI: 10.1016/s0140-6736(11)61091-x

- [7] Kim MK, Hara H. Current status of corneal xenotransplantation. *International Journal of Surgery*. 2015;**23**(Pt B):255-260. DOI: 10.1016/j.ijssu.2015.07.685
- [8] Kim MK, Wee WR, Park CG, Kim SJ. Xenocorneal transplantation. *Current Opinion in Organ Transplantation*. 2011;**16**(2):231-236. DOI: 10.1097/MOT.0b013e328344870c
- [9] Hayashi R, Ishikawa Y, Sasamoto Y, Katori R, Nomura N, Ichikawa T, et al. Co-ordinated ocular development from human iPS cells and recovery of corneal function. *Nature*. 2016;**531**(7594):376-380. DOI: 10.1038/nature17000
- [10] Griffith M, Osborne R, Munger R, Xiong X, Doillon CJ, Laycock NL, et al. Functional human corneal equivalents constructed from cell lines. *Science*. 1999;**286**(5447):2169-2172
- [11] Carlsson DJ, Li F, Shimmura S, Griffith M. Bioengineered corneas: How close are we? *Current Opinion in Ophthalmology*. 2003;**14**(4):192-197
- [12] Griffith M, Harkin DG. Recent advances in the design of artificial corneas. *Current Opinion in Ophthalmology*. 2014;**25**(3):240-247. DOI: 10.1097/icu.0000000000000049
- [13] Lee JJ, Kim DH, Jang YE, Choi HJ, Kim MK, Wee WR. The attitude toward xenocorneal transplantation in wait-listed subjects for corneal transplantation in Korea. *Xenotransplantation*. 2014;**21**(1):25-34. DOI: 10.1111/xen.12069
- [14] Kissam RS. Ceratoplasty in man. *New York Medical Journal*. 1844;**2**:281-282
- [15] Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL, Im GS, et al. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science*. 2002;**295**(5557):1089-1092. DOI: 10.1126/science.1068228 [doi]1068228 [pii]
- [16] Cooper DK. Clinical xenotransplantation—How close are we? *Lancet*. 2003;**362**(9383):557-559. DOI: S0140-6736(03)14118-9
- [17] Mou L, Chen F, Dai Y, Cai Z, Cooper DK. Potential alternative approaches to xenotransplantation. *International Journal of Surgery*. 2015;**23**(Pt B):322-326. DOI: 10.1016/j.ijssu.2015.06.085
- [18] Reardon S. New life for pig-to-human transplants. *Nature*. 2015;**527**(7577):152-154. DOI: 10.1038/527152a
- [19] Michel SG, Madariaga ML, Villani V, Shanmugarajah K. Current progress in xenotransplantation and organ bioengineering. *International Journal of Surgery*. 2015;**13**:239-244. DOI: 10.1016/j.ijssu.2014.12.011
- [20] Perkel JM. Xenotransplantation makes a comeback. *Nature Biotechnology*. 2016;**34**(1):3-4. DOI: 10.1038/nbt0116-3
- [21] Yang L, Guell M, Niu D, George H, Lesha E, Grishin D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015;**350**(6264):1101-1104. DOI: 10.1126/science.aad1191

- [22] Zhiqiang P, Cun S, Ying J, Ningli W, Li W. WZS-pig is a potential donor alternative in corneal xenotransplantation. *Xenotransplantation*. 2007;**14**(6):603-611. DOI: XEN432 [pii]10.1111/j.1399-3089.2007.00432.x [doi]
- [23] Choi HJ, Kim MK, Lee HJ, Ko JH, Jeong SH, Lee JI, et al. Efficacy of pig-to-rhesus lamellar corneal xenotransplantation. *Investigative Ophthalmology & Visual Science*. 2011;**52**(9):6643-6650. DOI: 10.1167/iovs.11-7273
- [24] Choi HJ, Lee JJ, Kim DH, Kim MK, Lee HJ, Ko AY, et al. Blockade of CD40-CD154 costimulatory pathway promotes long-term survival of full-thickness porcine corneal grafts in nonhuman primates: Clinically applicable xenocorneal transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2015;**15**(3):628-641. DOI: 10.1111/ajt.13057
- [25] Kim MK, Choi HJ, Kwon I, Pierson 3rd RN, Cooper DK, Soullillou JP, et al. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of xenocorneal transplantation. *Xenotransplantation*. 2014;**21**(5):420-430. DOI: 10.1111/xen.12129
- [26] Zhang MC, Liu X, Jin Y, Jiang DL, Wei XS, Xie HT. Lamellar keratoplasty treatment of fungal corneal ulcers with acellular porcine corneal stroma. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2015;**15**(4):1068-1075. DOI: 10.1111/ajt.13096
- [27] Nishida T, Saika S. Cornea and Sclera: Anatomy and Physiology. In *Cornea* (Krachmer, Mannis, and Holland). 3rd Ed. MOSBY Elsevier Inc. New York. 2011;3-24
- [28] Hori J, Vega JL, Masli S. Review of ocular immune privilege in the year 2010: Modifying the immune privilege of the eye. *Ocular Immunology and Inflammation*. 2010;**18**(5):325-333. DOI: 10.3109/09273948.2010.512696
- [29] Treacy O, Fahy G, Ritter T, O'Flynn L. Corneal immunosuppressive mechanisms, anterior chamber-associated immune deviation (ACAID) and their role in allograft rejection. *Methods in Molecular Biology*. 2016;**1371**:205-214. DOI: 10.1007/978-1-4939-3139-2_13
- [30] Vanhove B, Renard JP, Soullillou JP. Genetic engineering in the pig. Gene knockout and alternative techniques. *Annals of the New York Academy of Sciences*. 1998;**862**:28-36
- [31] Amano S, Shimomura N, Kaji Y, Ishii K, Yamagami S, Araie M. Antigenicity of porcine cornea as xenograft. *Current Eye Research Journal*. 2003;**26**(6):313-318
- [32] Lee HI, Kim MK, Oh JY, Ko JH, Lee HJ, Wee WR, et al. Gal alpha(1-3)Gal expression of the cornea in vitro, in vivo and in xenotransplantation. *Xenotransplantation*. 2007;**14**(6):612-618. DOI: XEN433 [pii]10.1111/j.1399-3089.2007.00433.x

- [33] Kim YG, Oh JY, Gil GC, Kim MK, Ko JH, Lee S, et al. Identification of alpha-Gal and non-Gal epitopes in pig corneal endothelial cells and keratocytes by using mass spectrometry. *Current Eye Research Journal*. 2009;**34**(10):877-895. DOI: 10.3109/02713680903184243 [pii]10.3109/02713680903184243
- [34] Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science*. 2003;**299**(5605):411-414
- [35] Cohen D, Miyagawa Y, Mehra R, Lee W, Isse K, Long C, et al. Distribution of non-gal antigens in pig cornea: Relevance to corneal xenotransplantation. *Cornea*. 2014;**33**(4):390-397. DOI: 10.1097/ico.0000000000000069
- [36] Hara H, Koike N, Long C, Piluek J, Roh DS, SundarRaj N, et al. Initial in vitro investigation of the human immune response to corneal cells from genetically engineered pigs. *Investigative Ophthalmology & Visual Science*. 2011;**52**(8):5278-5286. DOI: 10.1167/iovs.10-6947
- [37] Ezzelarab M, Ayares D, Cooper DK. Carbohydrates in xenotransplantation. *Immunology and Cell Biology*. 2005;**83**(4):396-404. DOI: 10.1111/j.1440-1711.2005.01344.x
- [38] Yeh P, Ezzelarab M, Bovin N, Hara H, Long C, Tomiyama K, et al. Investigation of potential carbohydrate antigen targets for human and baboon antibodies. *Xenotransplantation*. 2010;**17**(3):197-206. DOI: 10.1111/j.1399-3089.2010.00579.x
- [39] Lutz AJ, Li P, Estrada JL, Sidner RA, Chihara RK, Downey SM, et al. Double knockout pigs deficient in N-glycolylneuraminic acid and galactose alpha-1,3-galactose reduce the humoral barrier to xenotransplantation. *Xenotransplantation*. 2013;**20**(1):27-35. DOI: 10.1111/xen.12019
- [40] Lee W, Miyagawa Y, Long C, Ekser B, Walters E, Ramsoondar J, et al. Expression of NeuGc on pig corneas and its potential significance in pig corneal xenotransplantation. *Cornea*. 2016;**35**(1):105-113
- [41] Lee W, Hara H, Ezzelarab MB, Iwase H, Bottino R, Long C, et al. Initial in vitro studies on tissues and cells from GTKO/CD46/NeuGcKO pigs. *Xenotransplantation*. 2016;**23**(2):137-150
- [42] Dong X, Hara H, Wang Y, Wang L, Zhang Y, Cooper DK, et al. Initial study of alpha1,3-galactosyltransferase gene-knockout/CD46 pig full-thickness corneal xenografts in rhesus monkeys. *Xenotransplantation*. 2017;**23**(3):202-210. DOI: 10.1111/xen.12282. 10.1111/xen.12234
- [43] Khaireddin R, Wachtlin J, Hopfenmuller W, Hoffmann F. HLA-A, HLA-B and HLA-DR matching reduces the rate of corneal allograft rejection. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2003;**241**(12):1020-1028. DOI: 10.1007/s00417-003-0759-9
- [44] Hargrave SL, Hay C, Mellon J, Mayhew E, Niederkorn JY. Fate of MHC-matched corneal allografts in Th1-deficient hosts. *Investigative Ophthalmology & Visual Science*. 2004;**45**(4):1188-1193

- [45] van Essen TH, Roelen DL, Williams KA, Jager MJ. Matching for human leukocyte antigens (HLA) in corneal transplantation—To do or not to do. *Progress in Retinal and Eye Research*. 2015;**46**:84-110. DOI: 10.1016/j.preteyeres.2015.01.001
- [46] Bothwell AL. Characterization of the human antiporcine immune response: A prerequisite to xenotransplantation. *Immunologic Research*. 1999;**19**(2-3):233-243. DOI: 10.1007/bf02786491
- [47] Plenter RJ, Grazia TJ, Doan AN, Gill RG, Pietra BA. CD4 T cells mediate cardiac xenograft rejection via host MHC Class II. *Journal of Heart and Lung Transplantation*. 2012;**31**(9):1018-1024
- [48] Yamada K, Sachs DH, DerSimonian H. Human anti-porcine xenogeneic T cell response. Evidence for allelic specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. *Journal of Immunology*. 1995;**155**(11):5249-5256
- [49] Taylor CJ, Tang KG, Smith SI, White DJ, Davies HF. HLA-specific antibodies in highly sensitized patients can cause a positive crossmatch against pig lymphocytes. *Transplantation*. 1998;**65**(12):1634-1641
- [50] Yamada K, Sachs DH, DerSimonian H. Human anti-porcine xenogeneic T cell response. Evidence for allelic specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. *Molecular Immunology*. 2010;**47**(4):809-815. DOI: S0161-5890(09)00760-3 [pii] 10.1016/j.molimm.2009.10.004
- [51] Kim MK, Oh JY, Ko JH, Lee HJ, Jung JH, Wee WR, et al. DNA microarray-based gene expression profiling in porcine keratocytes and corneal endothelial cells and comparative analysis associated with xeno-related rejection. *Journal of Korean Medical Science*. 2009;**24**(2):189-196. DOI: 10.3346/jkms.2009.24.2.189 [doi]
- [52] Reyes LM, Estrada JL, Wang ZY, Blosser RJ, Smith RF, Sidner RA, et al. Creating class I MHC-null pigs using guide RNA and the Cas9 endonuclease. *Journal of Immunology*. 2014;**193**(11):5751-5757. DOI: 10.4049/jimmunol.1402059
- [53] Lee JM, Tu CF, Yang PW, Lee KH, Tsuji K, Tsai MK, et al. Reduction of human-to-pig cellular response by alteration of porcine MHC with human HLA DPW0401 exogenes. *Transplantation*. 2002;**73**(2):193-197
- [54] Hara H, Witt W, Crossley T, Long C, Isse K, Fan L, et al. Human dominant-negative class II transactivator transgenic pigs—Effect on the human anti-pig T-cell immune response and immune status. *Immunology*. 2013;**140**(1):39-46. DOI: 10.1111/imm.12107
- [55] Hashimoto Y, Funamoto S, Sasaki S, Honda T, Hattori S, Nam K, et al. Preparation and characterization of decellularized cornea using high-hydrostatic pressurization for corneal tissue engineering. *Biomaterials*. 2010;**31**(14):3941-3948. DOI: S0142-9612(10)00170-5 [pii] 10.1016/j.biomaterials.2010.01.122
- [56] Zhou Y, Wu Z, Ge J, Wan P, Li N, Xiang P, et al. Development and characterization of acellular porcine corneal matrix using sodium dodecylsulfate. *Cornea*. 2011;**30**(1):73-82. DOI: 10.1097/ICO.0b013e3181dc8184 10.1016/j.biomaterials.2010.01.122.

- [57] Oh JY, Kim MK, Lee HJ, Ko JH, Wee WR, Lee JH. Processing porcine cornea for biomedical applications. *Tissue Engineering Part C Methods*. 2009. DOI: 10.1089/ten.TEC.2009.0022 [doi]
- [58] Lee W, Miyagawa Y, Long C, Cooper DK, Hara H. A comparison of three methods of decellularization of pig corneas to reduce immunogenicity. *International Journal of Ophthalmology*. 2014;7(4):587-593. DOI: 10.3980/j.issn.2222-3959.2014.04.01
- [59] Hashimoto Y, Funamoto S, Sasaki S, Negishi J, Honda T, Hattori S, et al. Corneal regeneration by deep anterior lamellar keratoplasty (DALK) using decellularized corneal matrix. *PLoS One*. 2015;10(7):e0131989
- [60] Wu Z, Zhou Y, Li N, Huang M, Duan H, Ge J, et al. The use of phospholipase A(2) to prepare acellular porcine corneal stroma as a tissue engineering scaffold. *Biomaterials*. 2009;30(21):3513-3522. DOI: S0142-9612(09)00268-3 [pii] 10.1016/j.biomaterials.2009.03.003
- [61] He YG, Ross J, Niederkorn JY. Promotion of murine orthotopic corneal allograft survival by systemic administration of anti-CD4 monoclonal antibody. *Investigative ophthalmology & visual science*. 1991;32(10):2723-2728
- [62] Yamada J, Kurimoto I, Streilein JW. Role of CD4+ T cells in immunobiology of orthotopic corneal transplants in mice. *Investigative ophthalmology & visual science*. 1999;40(11):2614-2621
- [63] Qian Y, Dana MR. Molecular mechanisms of immunity in corneal allotransplantation and xenotransplantation. *Expert Rev Mol Med*. 2001;3(18):1-21. Doi: 10.1017/S1462399401003246
- [64] Niederkorn JY, Stevens C, Mellon J, Mayhew E. CD4+ T-cell-independent rejection of corneal allografts. *Transplantation*. 2006;81(8):1171-1178. DOI: 10.1097/01.tp.0000203140.70742.cb00007890-200604270-00015 [pii]
- [65] Gebhardt BM, Shi W. Experimental corneal allograft rejection. *Immunologic Research*. 2002;25(1):1-26. DOI: IR:25:1:01 [pii] 10.1385/IR:25:1:01
- [66] Hargrave SL, Taherzadeh S, Hegde S, Niederkorn J. High-risk corneal allografts are capable of stimulating complement dependent cytolytic antibodies. *Cornea*. 2000;19(4):521-525
- [67] Hegde S, Mellon JK, Hargrave SL, Niederkorn JY. Effect of alloantibodies on corneal allograft survival. *Investigative Ophthalmology & Visual Science*. 2002;43(4):1012-1018
- [68] Ross JR, Howell DN, Sanfilippo FP. Characteristics of corneal xenograft rejection in a discordant species combination. *Investigative Ophthalmology & Visual Science*. 1993;34(8):2469-2476
- [69] Ross JR, Sanfilippo FP, Howell DN. Histopathologic features of rejecting orthotopic corneal xenografts. *Current Eye Research*. 1994;13(10):725-730
- [70] Tanaka K, Yamada J, Streilein JW, Sonoda K, Higuchi R. Xenoreactive CD4+ T cells and acute rejection of orthotopic guinea pig corneas in mice. *Investigative Ophthalmology & Visual Science*. 2000;41(7):1827-1832

- [71] Tanaka K, Sonoda K, Streilein JW. Acute rejection of orthotopic corneal xenografts in mice depends on CD4(+) T cells and self-antigen-presenting cells. *Investigative Ophthalmology & Visual Science*. 2001;**42**(12):2878-2884
- [72] Holan V, Vitova A, Krulova M, Zajicova A, Neuwirth A, Filipec M, et al. Susceptibility of corneal allografts and xenografts to antibody-mediated rejection. *Immunology Letters*. 2005;**100**(2):211-213. DOI: 10.1016/j.imlet.2005.03.013
- [73] Oh JY, Kim MK, Ko JH, Lee HJ, Kim Y, Park CS, et al. Acute cell-mediated rejection in orthotopic pig-to-mouse corneal xenotransplantation. *Xenotransplantation*. 2009;**16**(2):74-82. DOI: XEN514 [pii] 10.1111/j.1399-3089.2009.00514.x
- [74] Oh JY, Kim MK, Lee HJ, Ko JH, Kim Y, Park CS, et al. Complement depletion with cobra venom factor delays acute cell-mediated rejection in pig-to-mouse corneal xenotransplantation. *Xenotransplantation*. 2010;**17**(2):140-146. DOI: XEN574 [pii] 10.1111/j.1399-3089.2010.00574.x
- [75] Choi HJ, Kim MK, Lee HJ, Jeong SH, Kang HJ, Park CS, et al. Effect of alphaGal on corneal xenotransplantation in a mouse model. *Xenotransplantation*. 2011;**18**(3):176-182. DOI: 10.1111/j.1399-3089.2011.00641.x
- [76] Vabres B, Le Bas-Bernardet S, Riochet D, Cherel Y, Minault D, Hervouet J, et al. hCTLA4-Ig transgene expression in keratocytes modulates rejection of corneal xenografts in a pig to non-human primate anterior lamellar keratoplasty model. *Xenotransplantation*. 2014;**21**(5):431-443. DOI: 10.1111/xen.12107
- [77] Kim DH, Kim J, Jeong HJ, Lee HJ, Kim MK, Wee WR. Biophysico-functional compatibility of Seoul National University (SNU) miniature pig cornea as xenocorneal graft for the use of human clinical trial. *Xenotransplantation*. 2016;**23**(3):202-210. DOI: 10.1111/xen.12234
- [78] Lee SE, Mehra R, Fujita M, Roh DS, Long C, Lee W, et al. Characterization of porcine corneal endothelium for xenotransplantation. *Seminars in Ophthalmology*. 2014;**29**(3):127-135. DOI: 10.3109/08820538.2013.787104
- [79] Fujita M, Mehra R, Lee SE, Roh DS, Long C, Funderburgh JL, et al. Comparison of proliferative capacity of genetically-engineered pig and human corneal endothelial cells. *Ophthalmic Research*. 2013;**49**(3):127-138. DOI: 10.1159/000342978
- [80] Doughty MJ, Zaman ML. Human corneal thickness and its impact on intraocular pressure measures: A review and meta-analysis approach. *Survey of Ophthalmology*. 2000;**44**(5):367-408
- [81] Faber C, Scherfig E, Prause JU, Sorensen KE. Corneal thickness in pigs measured by ultrasound pachymetry in vivo. *Scandinavian Journal of Laboratory Animal Science*. 2008;**35**:39-43
- [82] Lee HI, Kim MK, Ko JH, Lee HJ, Lee JH, Wee WR. The characteristics of porcine cornea as a xenograft. *Journal of the Korean Ophthalmological Society*. 2006;**47**(12):2020-2029

- [83] Sanchez I, Martin R, Ussa F, Fernandez-Bueno I. The parameters of the porcine eyeball. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2011;**249**:475-482
- [84] Zeng Y, Yang J, Huang K, Lee Z, Lee X. A comparison of biomechanical properties between human and porcine cornea. *Journal of Biomechanics*. 2001;**34**(4):533-537
- [85] Shufelt C, Fraser-Bell S, Ying-Lai M, Torres M, Varma R. Refractive error, ocular biometry, and lens opalescence in an adult population: The Los Angeles Latino Eye Study. *Investigative Ophthalmology & Visual Science*. 2005;**46**(12):4450-4460. DOI: 10.1167/iovs.05-0435
- [86] Snelligen T, Rao GN, Shrestha JK, Huq F, Cheng H. Quantitative and morphological characteristics of the human corneal endothelium in relation to age, gender, and ethnicity in cataract populations of South Asia. *Cornea*. 2001;**20**(1):55-58
- [87] Kim MK, Lee JJ, Choi HJ, Kwon I, Lee H, Song JS, et al. Ethical and regulatory guidelines in clinical trials of xenocorneal transplantation in Korea; the Korean xenocorneal transplantation consensus statement. *Xenotransplantation*. 2013;**20**(4):209-218. DOI: 10.1111/xen.12036
- [88] Kelley CG, Yamaguchi T, Santana E, Kaufman HE. A primate model of human corneal transplantation. *Investigative Ophthalmology & Visual Science*. 1984;**25**(9):1061-1064
- [89] Epstein AJ, de Castro TN, Laibson PR, Cohen EJ, Rapuano CJ. Risk factors for the first episode of corneal graft rejection in keratoconus. *Cornea*. 2006;**25**(9):1005-1011. DOI: 10.1097/01.ico.0000240098.01468.4c 00003226-200610000-00002 [pii]
- [90] Boisjoly HM, Bernard PM, Dube I, Laughrea PA, Bazin R, Bernier J. Effect of factors unrelated to tissue matching on corneal transplant endothelial rejection. *American Journal of Ophthalmology*. 1989;**107**(6):647-654
- [91] Panda A, Vanathi M, Kumar A, Dash Y, Priya S. Corneal graft rejection. *Survey of Ophthalmology*. 2007;**52**(4):375-396. DOI: 10.1016/j.survophthal.2007.04.008. S0039-6257(07)00056-2 [pii]
- [92] Rose AG, Cooper DK, Human PA, Reichenspurner H, Reichart B. Histopathology of hyperacute rejection of the heart: Experimental and clinical observations in allografts and xenografts. *Journal of Heart and Lung Transplantation*. 1991;**10**(2):223-234
- [93] Lee HI, Kim MK, Oh JY, Ko JH, Lee HJ, Wee WR, et al. The role of cyclosporine and mycophenolate in an orthotopic porcine-to-rat corneal xenotransplantation. *Journal of the Korean Ophthalmological Society*. 2008;**23**(3):492-501. DOI: 10.3346/jkms.2008.23.3.492 200806492 [pii]
- [94] Lee JK, Ryu YH, Ahn JI, Kim MK, Lee TS, Kim JC. The effect of lyophilization on graft acceptance in experimental xenotransplantation using porcine cornea. *Artificial Organs*. 2010;**34**(1):37-45. DOI: 10.1111/j.1525-1594.2009.00789.x
- [95] Oh JY, Kim MK, Ko JH, Lee HJ, Park CG, Kim SJ, et al. Histological differences in full-thickness vs. lamellar corneal pig-to-rabbit xenotransplantation. *Veterinary Ophthalmology*. 2009;**12**(2):78-82. DOI: 10.1111/j.1463-5224.2008.00680.x VOP680 [pii]

- [96] Cooper DK, Satyananda V, Eksler B, van der Windt DJ, Hara H, Ezzelarab MB, et al. Progress in pig-to-non-human primate transplantation models (1998-2013): A comprehensive review of the literature. *Xenotransplantation*. 2014;**21**(5):397-419. DOI: 10.1111/xen.12127
- [97] Li A, Pan Z, Jie Y, Sun Y, Luo F, Wang L. Comparison of immunogenicity and porcine-to-rhesus lamellar corneal xenografts survival between fresh preserved and dehydrated porcine corneas. *Xenotransplantation*. 2011;**18**(1):46-55. DOI: 10.1111/j.1399-3089.2011.00626.x
- [98] Jie Y, Liu L, Pan Z, Wang L. Survival of pig-to-rhesus corneal xenografts prolonged by prior donor bone marrow transplantation. *Molecular Medicine Reports*. 2013;**7**(3):869-874. DOI: 10.3892/mmr.2013.1294
- [99] Kim J, Kim DH, Choi HJ, Lee HJ, Kang HJ, Park CG, Hwang ES, Kim MK, Wee WR. Anti-CD40 antibody-mediated costimulation blockade promotes long-term survival of deep-lamellar porcine corneal grafts in non-human primates. *Xenotransplantation*. 2017 Apr 10
- [100] Sykes M, d'Apice A, Sandrin M. Position paper of the Ethics Committee of the International Xenotransplantation Association. *Transplantation*. 2004;**78**(8):1101-1107. DOI: 00007890-200410270-00003 [pii]
- [101] Hering BJ, Cooper DK, Cozzi E, Schuurman HJ, Korbitt GS, Denner J, et al. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Executive summary. *Xenotransplantation*. 2009;**16**(4):196-202. DOI: 10.1111/j.1399-3089.2009.00547.x XEN547 [pii]
- [102] Denner J, Schuurman HJ, Patience C. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 5: Strategies to prevent transmission of porcine endogenous retroviruses. *Xenotransplantation*. 2009;**16**(4):239-248. DOI: 10.1111/j.1399-3089.2009.00544.x XEN544 [pii]
- [103] Schuurman HJ. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2: Source pigs. *Xenotransplantation*. 2009;**16**(4):215-222. DOI: 10.1111/j.1399-3089.2009.00541.x
- [104] Williams KA, Coster DJ. The immunobiology of corneal transplantation. *Transplantation*. 2007;**84**(7):806-813. DOI: 10.1097/01.tp.0000285489.91595.13
- [105] Reinhard T, Reis A, Bohringer D, Malinowski M, Voiculescu A, Heering P, et al. Systemic mycophenolate mofetil in comparison with systemic cyclosporin A in high-risk keratoplasty patients: 3 years' results of a randomized prospective clinical trial. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2001;**239**(5):367-372
- [106] Williams KA, Esterman AJ, Bartlett C, Holland H, Hornsby NB, Coster DJ. How effective is penetrating corneal transplantation? Factors influencing long-term outcome in multivariate analysis. *Transplantation*. 2006;**81**(6):896-901. DOI: 10.1097/01.tp.0000185197.37824.35

- [107] Denner J, Tonjes RR, Takeuchi Y, Fishman J, Scobie L. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes-Chapter 5: Recipient monitoring and response plan for preventing disease transmission. *Xenotransplantation*. 2016;**23**(1):53-59. DOI: 10.1111/xen.12227
- [108] Spizzo T, Denner J, Gazda L, Martin M, Nathu D, Scobie L, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes-Chapter 2a: Source pigs-preventing xenozyoonoses. *Xenotransplantation*. 2016;**23**(1):25-31. DOI: 10.1111/xen.12223
- [109] Rayat GR, Gazda LS, Hawthorne WJ, Hering BJ, Hosking P, Matsumoto S, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 3: Porcine islet product manufacturing and release testing criteria. *Xenotransplantation*. 2016;**23**(1):38-45. DOI: 10.1111/xen.12225
- [110] Hering BJ, Cozzi E, Spizzo T, Cowan PJ, Rayat GR, Cooper DK, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Executive summary. *Xenotransplantation*. 2016;**23**(1):3-13. DOI: 10.1111/xen.12231
- [111] Hering BJ, O'Connell PJ. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 6: Patient selection for pilot clinical trials of islet xenotransplantation. *Xenotransplantation*. 2016;**23**(1):60-76. DOI: 10.1111/xen.12228
- [112] Cowan PJ, Ayares D, Wolf E, Cooper DK. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2b: Genetically modified source pigs. *Xenotransplantation*. 2016;**23**(1):32-37. DOI: 10.1111/xen.12224
- [113] Cozzi E, Tonjes RR, Gianello P, Buhler LH, Rayat GR, Matsumoto S, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 1: Update on national regulatory frameworks pertinent to clinical islet xenotransplantation. *Xenotransplantation*. 2016;**23**(1):14-24. DOI: 10.1111/xen.12222
- [114] Cooper DK, Bottino R, Gianello P, Graham M, Hawthorne WJ, Kirk AD, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 4: Pre-clinical efficacy and complication data required to justify a clinical trial. *Xenotransplantation*. 2016;**23**(1):46-52. DOI: 10.1111/xen.12226
- [115] Morozov VA, Wynyard S, Matsumoto S, Abalovich A, Denner J, Elliott R. No PERV transmission during a clinical trial of pig islet cell transplantation. *Virus Research*. 2017;**227**:34-40. DOI: 10.1016/j.virusres.2016.08.012