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Original Article

Ocular surface rehabilitation Application of human amniotic membrane in high-risk penetrating keratoplasties

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Abstract

Background: Human amniotic membrane is a versatile tool for management of ocular surface disorders. This study evaluates the effect of cryopreserved human amniotic membrane (hAM) on one-year survival of penetrating keratoplasties (PKP) in high-risk recipients.
Method: This is a retrospective noncomparative cohort study of 58 consecutive eyes undergoing PKP with concurrent placement of a self-retained cryopreserved hAM (PROKERA®) at a tertiary care center from January 2009 to July 2010.
Results: Mean patient age was 66.7 ± 17.2 years and 30 (54%) were males. 51 eyes were pseudophakic and one aphakic. 27 eyes were glaucomatous; 24 had glaucoma drainage device and 2 had previous endocyclophotocoagulation. 12 patients had PKP for the first time and 46 had repeat PKP (average number of prior PKP = 1.63 ± 1.1, range: 1–5).
Risk factors for graft failure included repeat PKP (79.3%), corneal neovascularization (51.7%), preexisting glaucoma (46.6%), and presence of anterior synechiae (37.9%). Both First Transplant and Repeat Transplant groups had similar survival rates until 6 months after transplant (75% vs 74%, odds ratio = 1.06, $p = 1.00$). At 12 months, First Transplant group showed a better survival rate (67% vs 43%, odds ratio = 2.60, $p = 0.20$). Eyes with >3 risk factors had a higher graft failure rate (odds ratio = 5.81, $p = 0.003$).
Conclusion: Survey of the literature suggests that high-risk PKP with concurrent hAM placement demonstrate comparable graft survival. Presence of multiple risk factors is associated with poor survival.

Keywords: Penetrating keratoplasty, Graft rejection, Immunomodulation, Anti-angiogenesis, Amniotic membrane, ProKera

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Introduction

The human amniotic membrane (hAM) has become a versatile tool in the management of ocular surface disorders. It is the inner most layer of the placenta, consisting of the maternal outer chorion and the fetal inner amnion. The hAM is comprised of a monolayer epithelium, a basement membrane, and an avascular stromal matrix. Initial indication was reported by De Rotth in 1940 for repair of conjunctival defects;[1] in the late 20th and early 21st centuries, the indications for hAM transplant rapidly expanded, as investigators discovered that the immunologically naïve hAM plays important roles in wound healing.

The unique properties of hAM are amply documented in the literature. It has been demonstrated to reduce the inflammatory response, induce suppression of interleukin alpha and interleukin 1 beta in epithelial cells,[2–6] support survival of the transplanted limbal epithelial stem cells via growth factors,[7]

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and via clearance of polymorphonuclear cells and inhibition of proteinase activity.[8,9] Induction of apoptosis of T lymphocytes and modulation of activated macrophages by hAM were found to play important roles in tissue remodeling.[10–14] Insults to the ocular surface, such as surgeries, trauma, or burns, may potentiate deleterious cascades resulting in decreased vision and patient comfort. The hAM has been shown to reduce scar tissue formation by trapping and preventing polymorphonuclear infiltration into the corneal stroma[15] and by downregulation of the transforming growth factor beta signaling system and myofibroblast differentiation of normal fibroblasts.[16,17] Other groups explored the utility of hAM as a bioactive substrate in limbal stem cell expansion and transplantation with varying results.[18–23] Interestingly, the anti-inflammatory activities and modulation of macrophages have been replicated in water-soluble hAM extract,[24] cryopreserved hAM tissue,[25] and a covalent complex of hyaluronan and the heavy chain of inter-α-inhibitor purified from hAM extract,[26] greatly expanding the versatility of hAM.

Presently, there are various commercially available preserved hAMs for ophthalmic applications. Bio Tissue Inc. (Doral, FL, USA) provides AmnioGraft®, AmnioGuard™, and PROKERA® via cryopreservation method. IOP Ophthalmics Inc. (Costa Mesa, CA, USA) supplies the free-dried alternatives, Ambio2™, Ambio5®, and AmbioDisk™. Recently, lyophilized extract of the fresh hAM (AMX®), prepared by Keera srl and distributed by Treviso Tissue Bank, Italy, has been made available in Europe. However, the literature on AMX® efficacy is rather scant.[27] Table 1 compares these tissues.

Although utility of hAM is widely reported for many ocular surface applications,[28,29] its use in corneal transplant is limited. Despite the fact that corneal transplantation is now the most successful human organ transplantation, routinely performed without HLA typing or systemic immunosuppression, the long-term success of penetrating keratoplasty (PKP) is dictated by risk factors.[30–38] Having multiple risk factors, such as preoperative diagnosis of corneal opacity, corneal neovascularization, presence of anterior synechiae, prior rejection, coexisting glaucoma, and recipient gender and age at time of transplant, predisposes the patient to graft rejection and failure.[39–50] Data from the literature suggest that topical steroids may be inadequate for high-risk recipients, systemic steroids and immunomodulatory therapies pose significant adverse effects, and newer therapeutic strategies are being explored to improve survival of high-risk transplants.[51–60]

The unique properties of hAM suggest that it may be an adjuvant therapy to reduce the risks of graft rejection in high-risk PKP. Accordingly, we set forth to investigate whether high-risk PKP recipients would benefit from hAM placement. In this retrospective series, we evaluate the failure rates of PKP when performed in conjunction with cryopreserved hAM placement in patients with high-risk features and compare our graft failure rates to those reported in the literature.[30,34,44–50]

Materials and methods

The study population consisted of 58 eyes of 56 patients who underwent PKP with placement of self-retained hAM devices (PROKERA®, Bio-Tissue, Inc., Doral, FL). PROKERA® is a class II medical device comprised of cryopreserved hAM clipped into a dual polymethyl methacrylate symblepharon ring system, where the stromal aspect is in contact with the corneal epithelium. It was selected because its placement can be performed without sutures and bioadhesives, reducing confounding factors. A retrospective chart review was conducted for demographics and pre- and post-operative findings. The main outcome measure was clinical determination of graft failure. Study protocol was approved by the Institutional Review Board; and strict adherence to the principles of the Declaration of Helsinki was followed.

All surgeries and preoperative/postoperative cares were performed by a single surgeon (SCY). Briefly, the donor button was prepared using a Barron punch and coated with viscoelastic device for protection. A Hessburg-Barron vacuum trephine and corneal scissors were used to excise the host corneal button. The graft was secured to the host bed with interrupted 10-0 nylon sutures; subsequently, ophthalmic viscoelastic device was irrigated and the anterior chamber was reformed with balanced salt solution. All suture knots were buried. Subconjunctival injections of cefazolin, tobramycin, and dexamethasone were given. Thereafter, a self-retained amniotic membrane device was placed over the corneal button. Postoperative care for these patients was provided by the same surgeon, and included standard regimen of antibiotic and steroid drops, as well as follow-up visits at postoperative (PO) day 1, PO week (POW) 1, PO month (POM) 1, POM 3, 6, 9 and 12.

Inclusion criteria were eyes with at least 12-month postoperative follow-up visits and having two or more risk factors for graft rejection and failure,[5–9,36] identified preoperatively. Risk factors are high-risk indications for transplant, corneal stromal neovascularization of two or more quadrants, history

Table 1. Comparison of commercially available preserved human amniotic membrane for ophthalmic applications.

Company	IOP Ophthalmics, Inc.	Bio Tissue, Inc.	Keera, srl
Preparation	De-epithelialized, dehydrated, sterilized with irradiation	Epithelialized, cryopreserved	Lyophilized extract of the fresh human amniotic membrane
Storage	Stored free-standing at 10–27 °C, good for 2 years	Attached to nitrocellulose paper, cryopreserved at −80 °C, good for 2 years	Soluble powder form, stored in dry environment at 18–20 °C
Activation	Activated with saline solution or bioadhesive agent	Thaw to room temperature before use	Dilution with sterile BSS
Delivery/ fixation method	Bioadhesive or suture fixation for Ambio™, contact lens for Ambiodisk™	Bioadhesive or suture fixation for AmnioGraft® and AmnioGuard™, self-retained symblepharon ring for PROKERA®	Topical using eye drop dispenser
Availability	Worldwide	Worldwide	Europe

of anterior segment inflammation, presence of anterior synechiae, glaucomatous eyes on medication or had glaucoma drainage implant (GDI) placement, severe ocular surface disorders such as limbal stem cell deficiency, ocular cicatricial pemphigoid, severe dry eye, and age less than 40 years. High-risk indications for transplant were prior graft failure, pseudophakic or aphakic bullous keratopathy, corneal perforation, or herpetic keratitis with visually significant corneal scar. Only patients receiving surgeries during the period of June 2009 to July 2010 and having one-year follow-up were included.

Results

Fifty-eight eyes met the inclusion criteria; nine patients were excluded due to insufficient follow-up. Preoperative demographics are tabulated in Table 2. Mean age was 66.7 ± 17.2 years and 30 patients (51.7%) were male. 51 eyes (88%) were pseudophakic and one (1.8%) was aphakic. 27 eyes were glaucomatous; 24 of these had GDIs and 2 had endocyclophotocoagulation performed for poorly controlled glaucoma. Twelve eyes received corneal transplants for the first time (First Transplant). Forty-six eyes received repeat PKP (Repeat Transplant). The average number of prior PKP was 1.63 ± 1.1 (range: 1–5). Indications for initial PKP included corneal scar (4), endothelial failure (3), microbial keratitis with perforation or visually significant corneal scar (5). Corneal neovascularization of two or more quadrants was observed in 30 eyes (51.7%) and presence of anterior synechiae existed in 22 eyes (37.9%). The mean numbers of risk factors for graft rejection were 3.0 ± 0.74 for the first PKP group and 3.6 ± 1.02 for the repeat PKP group. Fifteen patients received another concurrent surgery at the time of PKP (Table 3).

Overall one-year survival rate was 52%. At postoperative month six, both the First Transplant and Repeat Transplant groups showed similar survival rates (75% versus 74%, odds ratio (OR) = 1.06, $p = 1.00$). At twelve months, the First Transplant group appeared to have a better survival (67%), compared to that of the Repeat Transplant group (43%). This advantage did not reach statistical significance using univariate analysis (OR = 2.60, $p = 0.20$).

Univariate analysis was performed to calculate the odds ratios of graft failure for selected risk factors (Table 4). No significantly increased risks of failure were observed when comparing First Transplant versus Repeat Transplant (OR = 0.38, $p = 0.20$). Recipient corneal neovascularization equal to or greater than 2 quadrants was associated with increased risk of rejection but statistical significance was not achieved (OR = 2.0, $p = 0.29$). However, the OR of graft rejection increased to 5.81 ($p = 0.003$) when eyes with three or less risks factors were compared to eyes with more than three risks factors. Overall, eyes receiving concurrent intraocular surgery as tabulated in Table 2 did not show increased risks of rejection (OR = 1.31, $p = 0.767$). Interestingly, eyes undergoing synechiolysis had a rejection rate of 83% and appeared to be associated with increased risk of rejection (OR = 5.20, $p = 0.197$).

Table 2. Patient demographics and preoperative clinical characteristics.

Patient characteristics	Count	Percentage (%)
Age (years)	66.4 ± 17.4	
Gender (male)	30	52
High-risk indications	58	100
Corneal neovascularization	30	52
Herpetic keratitis	7	12
Anterior segment inflammation	12	21
Anterior synechiae	22	38
Ocular surface disorder	10	17
Glaucoma	27	47
Age <40	4	7
Pseudophakia or aphakia	52	90
Prior vitreoretinal surgery	5	9

Table 3. Concurrent surgical interventions.

Additional intervention	# of Patients
Synechiolysis	5
Synechiolysis + intraocular lens placement	1
Cataract extraction/intraocular lens placement	3
Secondary intraocular lens placement or exchange	2
Iridoplasty	1
Anterior vitrectomy	1
Glaucoma drainage implant	1
Temporary keratoplasty, vitrectomy, glaucoma drainage implant, intraocular lens exchange	1

Discussion

This present study evaluates the role of human amniotic membrane for prevention of graft rejection in high-risk penetrating keratoplasties. The idea is to leverage the inflammatory modulation activities of hAM to suppress graft rejection, as it is the leading cause of graft failure.[34] High-risk features have been associated with a 30–56% risk of graft failure at 3 years.[46] Prior studies reported the poor prognostic value of vascularization of recipient bed.[37,40–42] Indeed, a meta-analysis of 19 studies encompassing 24,944 grafts concluded that presence of recipient neovascularization increases the risk of future graft failure by 30% and more than doubles the risks of graft rejection.[37] We also found a 60% failure rate in host bed having two or more quadrants of corneal neovascularization, which was elevated compared to non-neovascularized host. Other studies found that glaucoma, intraocular inflammation, younger age[35,37] or vitrectomy performed at time of transplant[38,48] were associated with increased risk of failure. Our study corroborated these findings but we lack statistical power due to small sample size of patients having many high-risk features.

As expected, failed PKP is a poor prognosticator. In a retrospective study, Bersudsky and colleagues published failure rates of 63% in first regrafts that increased to 75–100% in third and fourth regrafts.[42] Yildiz et al. followed 45 patients over 6 years and reported a five-year graft failure rate of 53–64% in third and fourth grafts.[36] More recently, Fasolo and colleagues calculated a failure rate of 42.8%, with a median time to failure of 2.3 years in regrafts related to allograft rejection.[38] In our study, 87% (26 of 30) of the eyes that developed graft failure had prior failed transplants. These high-risk patients were expected to have substantial neovascularization and likely immune activation resulting from previously failed transplants.[34] The lower survival rates in our cohort, 67% for first transplants and 43% for the regrafts, may be the result of a different patient population with higher number of risk factors, 3.0 ± 0.74 in First Transplant group and 3.6 ± 1.02 for the Repeat Transplant group. Indeed, univariate analysis substantiated that having more than three preoperative risk factors significantly decreased graft survival.

Table 4. 12-month failure rates for each individual risk factor.

Risk factors	Failure rate (%)	Odds ratio	*p*-Value	95% confidence interval
First transplant	33.3			
Repeat transplant	56.5	2.6	0.20	0.10–1.46
Glaucoma	55.6	1.3	0.61	0.47–3.76
Corneal neovascularization (⩾2 quadrants)	60.0	2.0	0.29	0.70–5.70
Herpetic corneal scar	14.3	0.1	0.048	0.01–1.13
Prior anterior uveitis	58.3	1.4	0.75	0.39–5.06
Presence of anterior synechiae	63.6	2.2	0.18	0.74–6.50
3+ risk factors	71.0	5.8	0.003	0.06–0.54
Concurrent intraocular surgery	46.7	1.3	0.77	0.40–4.27
Synechiolysis	83.3	5.4	0.20	0.59–49.5

Interestingly, having concurrent intraocular surgeries did not significantly increase the rate of graft rejection. However, synechiolysis was associated with increased risk of graft failure. It is likely that anterior synechiae is an indicator for prior intraocular inflammation, prior surgery, trabecular meshwork compromise, or anatomic predisposition, that translate to surgical difficulty, increased risks of complications, or altered anterior chamber-associated immune deviation. Iris synechiae to the graft or host-graft junction may further facilitate antigen presentation and sensitization.[43,46] Thus, the correlation between synechiolysis and graft rejection was not unexpected.

Many limitations are evident in this retrospective study. Two critical limitations were the lack of controls and inadequate power for meaningful statistical analysis. Additionally, our cohort consisted of high-risk population, as shown in Tables 2 and 3, which prohibited direct comparison with the data reported in the literature. Therefore, we were unable to draw a definitive conclusion regarding the benefits of adjunctive cryopreserved human amniotic membrane placement, in the form of the PROKERA® device, in high-risk penetrating keratoplasty. This might be related to the limited efficacy of hAM on the ocular surface in suppressing stromal or endothelial immunogenic cascades. Further studies are encouraged to definitely address the role of hAM in high-risk PKP.

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Conflict of interest

The authors declared that there is no conflict of interest.

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