Amniotic Membrane Transplantation for Ocular Surface Reconstruction†

PINNITA PRABHASAWAT, M.D.*,***, PANIDA KOSRIRUKVONGS, M.D.*,***,
WIPAWEE BOORANAPONG, M.D.*, YONGYUTRA VAJARADUL, M.D.**,***

Abstract

Objective : To study the efficacy of amniotic membrane transplantation in various indications for ocular surface reconstruction.

Method : Amniotic membrane transplantations were performed in 140 eyes (130 patients) for ocular surface reconstruction. The indications for the corneal group were limbal stem cell deficiency, bullous keratopathy, persistent epithelial defect, band keratopathy, prosthesis, corneal ulcer and acute chemical burn. The indications for the conjunctival group were grafts for pterygium, conjunctival tumors, symblepharon, and covering the scleral graft.

Results : Success was noted in 75.7 per cent (106/140) eyes, partial success in 17.9 per cent (25/140) eyes, and failure in 6.4 per cent (9/140) eyes for a mean follow-up of 6.6 months (1-19 months). The success and partial success rate were 80.6 per cent (54/67), 14.9 per cent (10/67) in the corneal group and 71.2 per cent (52/73), 20.6 per cent (15/73) in the conjunctival group.

Conclusion : Amniotic membrane transplantation can solve some difficult ocular surface problems, and can be used to promote epithelial healing, reduce inflammation and scarring.

Key word : Amniotic Membrane, Amniotic Membrane Transplantation, Corneal Surface reconstruction, Conjunctival Surface Reconstruction, Limbal Stem Cell Deficiency, Persistent Epithelial Defect, Conjunctival Tumor, Pterygium, Symblepharon

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* Department of Ophthalmology,
** Department of Orthopedics Surgery,
*** The Bangkok Biomaterial Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

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Human amniotic membrane has been used for many purposes since 1910[1]. It has been widely used effectively as a biological dressing for acute burn[2-5], skin ulcers[6,7] and abdominal wound[8] to relieve pain, promote epithelial healing and decrease infection rate of the wound. The amniotic membrane has also been used as a graft for burned skin[9,10], vaginal reconstruction in the absence of vagina[11,12], reconstruction in otolarynx[13], and repair of omphalocele[14]. It has also been reported to prevent tissue adhesion in surgeries of the head, abdominal and pelvic cavity[15-17].

In Ophthalmology, human fetal membrane has been used as grafts for conjunctival defect[18] and pterygium[19] since 1940. For reasons still not clear to us, clinical uses of amniotic membrane transplantation disappeared until 1993, when preserved human amniotic membrane was reintroduced by Kim and Tseng[20], who showed that corneal surface with total limbal deficiency can be reconstructed in rabbits. Since then the membrane has been widely used successfully for many purposes in ocular surface reconstruction. It has been shown to be effective in promoting epithelial healing in persistent epithelial defects[21-23], and successfully combines with limbal transplantation in Steven Johnson's syndrome and chemical burn [24-27]. It has also been successfully used as an alternative graft in pterygium excision[28,29], symblepharon and conjunctival tumors[30]. Besides, it has been reported to prevent fibrosis in trabeculectomy[31] and reduce haze in PRK[32, 33].

Based on these encouraging results of amniotic membrane transplantation (AMT), we report herein our experience in using this procedure in a variety of difficult ocular surface problems. We divided our patients into two groups for corneal surface and conjunctival surface reconstruction, respectively. We report their efficacy and limitation.

PATIENTS AND METHOD

Patients

This study was approved by the committee for protection of human subjects in research of the Faculty of Medicine, Siriraj Hospital, Mahidol University. From September, 1997 to April, 1999, amniotic membrane transplantation was performed in 130 patients (140 eyes) with various ocular surface disorders after obtaining their informed consent. The patients were divided into corneal and conjunctival surface groups. Clinical data, indications for surgery and goal of treatments in each group are summarized in Table 1-4.

The corneal surface reconstruction group was further divided into 2 subgroups. The first subgroup of 21 cases (27 eyes) showed limbal stem cell deficiency, caused by Steven-Johnson disease (13 cases, 18 eyes), chemical burn (7 cases, 8 eyes), and HSV keratitis (1 case, 1 eye). In this subgroup, 12 patients (12 eyes) received amniotic membrane transplantation (AMT) combined with limbal transplantation in the same or subsequent operation, while 7 patients (7 eyes) received autograft and 5 patients (5 eyes) received allograft limbal transplantation. Nine patients (13 eyes) received AMT alone because of their financial difficulties of obtaining systemic cyclosporin which is indispensable for allograft limbal transplantation. The second subgroup consisted of other corneal diseases such as bullous keratopathy (19 cases, 19 eyes), persistent epithelial defect, dellen and descemetocele (11 cases, 11 eyes), band keratopathy after removal of calcium deposit (7 cases, 7 eyes), preparing for prosthesis (1 case, 1 eye), corneal ulcer (1 case, 1 eye), and acute chemical burn (1 case, 1 eye).

In the conjunctival surface reconstruction, amniotic membrane was used as grafts for pterygium excision in 43 cases (47 eyes), following conjunctival tumor excision in 17 cases (17 eyes), in conjunction with symblepharonlysis in 7 cases (7 eyes), and for covering scleral graft in 2 cases (2 eyes).

Preparation of preserved human amniotic membrane

The placenta was obtained from elective caesarian section from mothers seronegative for human immunodeficiency virus (antigen and antibody), hepatitis B & C viruses and syphilis. Also negative for HIV, EBV, CMV and HSV by using PCR technique. Human placenta that met the above selection criteria was transferred to the laboratory of Bangkok Biomaterial Center in a sterile plastic bag stored in an ice bucket. The following procedures were identical to what has been reported[21,26,28,30] except normal saline was used instead of Eale's balanced salt solution (EBSS) to rinse the excessive blood clot. The

membranes were kept in -70°C for up to one year before use. After this preparation, sterility of the amniotic membrane was checked by culture for bacteria and fungus and all cultures were negative.

Amniotic membrane transplantation

After obtaining informed consent, the patient was subjected to the same routine admitting preoperative tests. One surgeon (PP) performed all surgeries using different techniques depending on the indications.

Amniotic membrane transplantation for limbal deficiency

After retrobulbar block with 2 per cent xylocain combined with 2 per cent marcain or general anesthesia, the abnormal scar and symblepharon was excised at the bulbar area until the fornix. Amniotic membrane was placed on the whole surface area of the bulbar part and the corneal surface. Running suture with 10.0 nylon was performed at the limbal area to fix the amniotic membrane to the sclera. Interrupted sutures with 8.0 vicryl or continuous running sutures 10.0 nylon were performed to fix the amniotic membrane to the sclera 5-7 mm away from the limbal area. The procedure was similar to that described by Tseng et al(26).

Amniotic membrane transplantation for bullous keratopathy and band keratopathy

After topical anesthesia with 2 per cent tetracain eye drop, the loose epithelium was removed with a blade in bullous keratopathy or epithelium and calcium deposit was removed with 3 per cent EDTA in band keratopathy. The amniotic membrane was placed to cover the corneal epithelial defect, which in general was up to 9 mm in diameter and close to the limbus. Running suture was performed near the edge of the membrane to ensure the tight approximation of the membrane to the denuded corneal surface. A bandage lens was applied in some cases until the epithelial defect had healed.

Amniotic membrane transplantation for persistent epithelial defect, dellen, acute burn, and corneal ulcer

After retrobulbar injection or topical anesthetic drop (2% tetracaine eye drop), the base of the epithelial defect was debrided, and covered with a piece of amniotic membrane, sutured to the edge of the defect with interrupted 10-0 nylon. A bandage lens was applied until the defect had healed. The procedure was similar to that previously described(21).

Amniotic membrane transplantation for conjunctival surface

After removal of the pterygium, conjunctival mass or scar tissue together with abnormal fibrovascular tissue, the amniotic membrane was placed to cover the bare sclera, and sutured to the edge of the conjunctiva and fixed with the scleral tissue with running suture 10.0 nylon or interrupted suture 8.0 vicryl. Attention was given to ensure the graft was tight and flattened. The procedure was similar to that reported previously(30).

Post-operative evaluation and follow-up

After surgery, all patients were examined on the following day and daily while they were in the hospital. After discharge, they were followuped at 1 week, 2 weeks and then monthly thereafter or as needed for as long as possible. The same post-operative evaluation was applied to all patients with special attention given to patient's symptoms with respect to pain, discomfort, irritation, signs of inflammation at the surgical sites, and healing of the area covered by amniotic membrane using fluorescein staining. The outcome of AMT was judged by patient's symptoms, resolution of original ocular surface problems, appearance of surgical sites, and recurrence of the disease. Complications were recorded for infection, dislodgment or dissolution of the membrane graft with or without a non-healing ulcer or increased inflammatory and/or immune response over the membrane graft. If identified as the inciting cause but untreatable, the membrane was removed. The sterility of the membrane was rechecked again by bacterial and fungal cultures performed at the time following surgery and all cultures were negative.

The clinical outcome was categorized into success, partial success, and failure based on the following criteria in Table 1. When all goals of surgery were achieved in terms of relief of pain and irritation, reduction of inflammation and scaring and promotion of epithelial healing, the outcome was defined as "success". When part of

Table 1. Criteria for success, partial success and failure in each category.

Indication for surgery	Success	Partial success	Failure
Limbal stem cell deficiency	Relief photophobia, pain, irritation, and inflammation Decrease keratinization Release symblepharon	Relief photophobia, pain, and irritation Decrease inflammation and keratinization Some fibrovascular tissue or slight recurrent symblepharon	Persistence of pain, irritation, and inflammation Recurrence of keratinization and symblepharon
PED, dellen, descemetocele	Healed epithelial defect	Incomplete healing	None healing
Bullous keratopathy	Relief pain and irritation	Transient relief of pain and irritation	Persistence of pain and irritation
Band keratopathy	Relief pain, irritation, Delayed calcium deposit	Transient relief pain, irritation Recurrence of calcium deposit	Persistence of pain, irritation Recurrence of calcium deposit
Preparing for prosthesis	Wearing prosthesis without pain	Decreased pain	Persistence of pain, unable to wear prosthesis
Corneal ulcer	Healed ulcer	Incomplete healing	None healing
Acute burn	Healed epithelial defect	Incomplete healing	None healing
Pterygium	No recurrence, no scarring, excellent cosmetic result	Some fibrovascular tissue invading the graft, but not into the cornea	Recurrence of pterygium (fibrovascular tissue invading to the cornea)
Conjunctival tumor	No scar and excellent cosmetic result No symblepharon	Some fibrovascular tissue invading the graft	Prominent scar or symblepharon
Symblepharon	No recurrence Decrease restriction	Some scar but better than pre-operation	Same as pre-operation
Scleral graft	Protect scleral graft from melting and loss	Amniotic membrane partially dissolved, exposed scleral graft	Membrane dissolved, Scleral graft melted

PED = persistent epithelial defect

Table 2. Clinical data and results.

Group	No of cases/eyes	Age	Sex (M/F)	follow-up time (months)	Results eyes					
					Success		Partial success		Failure	
					no	%	no	%	no	%
Total	130/140	49.0 (3-85)	62/68	6.6 (1-19)	106	75.7	25	17.9	9	6.4
Cornea	61/67	44.6 (3-85)	34/26	7.0 (1-19)	54	80.6	10	14.9	3	4.5
Conjunctiva	69/73	53.0 (5-84)	28/41	6.1 (1-19)	52	71.2	15	20.6	6	8.2

these goals were achieved, e.g., relief of pain but with some inflammation or fibrovascular tissue invading the graft, the outcome was defined as "partial success". When none of the surgical goals was met, the outcome was defined as "failure".

RESULTS

As shown in Table 2, the mean age of these 130 patients (62 men, 68 women) was 49.0 years (ranging from 3 to 85 years), and the mean follow-up time was 6.6 months (ranging from 1 to 19 months). Based on the criteria given in Table 1, success was obtained in 106 eyes (75.7%), 54 eyes (80.6%), and 52 eyes (71.2%), respectively, for the total, the corneal surface group and the conjunctival surface group, respectively (Table 2). Partial success was noted in 25 eyes (17.9%), 10 eyes (14.9%), and 15 eyes (20.6%), respectively, for the total, the corneal surface group and the conjunctival surface group, respectively. Failure was noted in 9 eyes (6.4%), 3 eyes (4.5%), and 6 eyes (8.2%), respectively, for the total, the corneal

Table 3. Results of corneal surface reconstruction in each indication.

Indications for treatments	No of cases/eyes	Follow-up time		Results eyes					
				Success		Partial success		Failure	
		months		no	%	no	%	no	%
Limbal deficiency	21/27	6.8	1-18	18	66.7	7	25.9	2	7.4
AMG c LT	12*/12	7.2	1-18	11	91.7	1	8.3	0	
AMG s LT	11*/15	5.6	2-13	7	46.7	6	40.0	2	13.3
Bullous Keratopathy	19/19	8.0	3-17	18	94.7	1	5.3	0	
PED, Dellen, Descemetocele	11/11	6.7	2-15	11	100	0		0	
Band Keratopathy	7/7	7.0	2-17	5	71.4	2	28.6	0	
Prosthesis	1/1	1	1	1	100	0		0	
Corneal ulcer	1/1	5	5	1	100	0		0	
Acute chemical burn	1/1	4	4	0		0		1	100

Note PED = persistent epithelial defect, AMG = amniotic membrane graft, LT = limbal transplantation
* Two patients had amniotic membrane transplantation combined with limbal transplantation in one eye and amniotic membrane transplantation alone in another eye.
Definition of success, partial success, and failure is given in Table 1

Table 4. Results of conjunctival surface reconstruction in each indication.

Indications for treatments	No of cases/eyes	Follow-up time (months)	Results eyes					
			Success		Partial success		Failure	
			no	%	no	%	no	%
Pterygium	43/47	5.1 (1-19)	37	78.8	8	17.0	2	4.2
Conjunctival mass	17/17	7.6 (1-18)	12	70.6	5	29.4	0	
- Nevus								
- CIN or SCC								
Symblepharon	7/7	6.8 (1-15)	2	28.6	2	28.6	3	42.8
Scleral graft	2/2	10.0 (3-17)	1	50	0		1	50

Note CIN = Conjunctival intraepithelial neoplasia, SCC = squamous cell carcinoma

surface group, and the conjunctival surface group, respectively. The details of the success rate in each group are shown in Table 3 and 4.

Corneal Surface Reconstruction

In limbal stem cell deficiency patients, 18 out of 27 eyes (66.7%) were rated as success, and these patients felt more comfortable without irritation or inflammation, and showed improved vision. Vision improved from hand motion to at least 20/200 in 4 eyes of AMT combined with limbal allograft transplantation and in 5 eyes of AMT combined with limbal autograft transplantaion. Vision improved from hand motion to at least 10/200 or more than 2 snellen lines in 6 eyes of AMT alone (Table 5). Seven out of 21 eyes (25.9%) were rated as partial success, with decreased inflammation but mild fibrovascular tissue invasion into the grafts or slight recurrence of symblepharon. Importantly, all these patients with partial success felt more comfortable and satisfied with the outcome to the extent they would like to have the other eye operated on. Two eyes (7.4%) were rated as failure. One of them showed recurrent conjunctival keratinization due to severe keratoconjunctivitis sicca. The other case still showed the same inflammation as before surgery. Intriguingly, all cases rated as partial success or failure suffered from Steven-Johnson syndrome.

In the corneal surface group, 18 out of 19 eyes with symptomatic bullous keratopathy were rated as success and became pain-free with a stable corneal surface after AMT. The partial success case was of traumatic bullous keratopathy, pain and irritation were relieved in the first 2 months after surgery but recurred thereafter, and required the use of a contact lens to relieve pain and irritation. Five out of seven eyes with band keratopathy, which were rated as success, felt much more comfortable without irritation or pain

Table 5. Details of surgeries and outcomes.

Indications for treatments	AMT area (extent)	Surgeries with AMT		ED healing (weeks)	Vision change (eyes)		Complications (eyes)	
Limbal deficiency	Whole cornea and	PKP	3	2.8	Same	2	Corneal ulcer (3 mo)	1
AMG c LT	conjunctiva extend to	Tarsorrhaphy	3	2.1	Improve : Fc to ≥ 5/200	1	Corneal perforation (9 mo)	1
	5-7 mm from the limbus	PO	5		≥ 20/200	2		
					≥ 20/70	7		
AMG s LT		PO	14	3.7	Same	9	Conjunctivitis	1
	Tarsorrhaphy	7			Improve ≥ 2 lines	6	Lid abscess	1
	Lid correction	2					Entropion	1
							AMG detached	1
							Corneal ulcer (1 mo)	1
							Corneal perforation (2, 3 mo)	2
Bullous Keratopathy	Cornea 9 mm	-		2.2	Same		Corneal ulcer (2 mo)	1
							AMG detached	1
PED, Dellen, Descemetocele	Cornea 5-9 mm	-		1.8	Same	9	No	
					Improve : Fc to 20/200	1		
					Worse : 20/200 to HM	1		
Band Keratopathy	Cornea 9 mm	EDTA		1.8	Same		No	
Prosthesis	Cornea 9 mm	-		1	Same		No	
Corneal ulcer	Cornea 4 mm keratectomy	Superficial		1	Improve ≥ 2 lines	1	No	
Acute chemical burn	Cornea 9 mm	-		≥ 12	Same		ED	
Pterygium	Conjunctiva 8-10 mm	-		1.1	Same	39	Dellen	
					Improve ≥ 2 lines	8		3
Conjunctival lesion	Conjunctiva 10-30 mm	-		1.6	Same		No	
Symblepharon	Conjunctiva 30 mm	MMC	2	1.8	Same	3	No	
					Improve ≥ 2 lines	3		
Scleral graft	Conjunctiva 20 mm	Scleral graft	2	2	Same		No	

Note In the complication, the immediate complications does not mention time in the table
AMG = amniotic membrane graft, LT = limbal transplantation, PO = punctal occlusion, PKP = penetrating keratoplasty, ED = epithelial defect,
EDTA= Ethylene diamine tetrachloroacetic acid

and no recurrence after removal of calcific band keratopathy with 3 per cent EDTA and the whole cornea was covered with the amniotic membrane. Two patients were rated as partial success due to recurrence of band keratopathy at post-operative 5 months and 7 months, respectively, but they did not have any irritation and remained pain-free.

All 11 cases with persistent epithelial defect, dellen or descemetocele were rated success and their corneal surfaces were healed after AMT. The causes for their surface breakdowns were diverse and included herpes simplex keratitis, corneal ulcer, lid abnormalities from tumor removal, corneal surface irregularity, post radiation, following corneal graft rejection and post phacoemulsification. The mean duration for epithelial defect to heal was 1.8 weeks (ranging from 1 to 4 weeks). Among them, 2 cases had failed with bandage contact lens treatment prior to the amniotic membrane graft. In the two cases with descemetocele and dellen, the corneal stromal thickness increased after the healing promoted by amniotic membrane graft.

In one case who suffered from irritation and pain that prevented this blind patient from wearing eye prosthesis, AMT was successful to eliminate such irritation and pain that he did not undergo conjunctival flap. In the case with superficial corneal ulcer following candidiasis superficial keratectomy and amniotic membrane graft successfully restored the corneal surface integrity. The epithelial defect and ulcer was completely healed in 1 week despite prior failure following medical treatment for more than 3 weeks. However, in one case with acute (2 weeks after burns) stage 4 burn with a 9 mm epithelial defect with severe ischemia, amniotic transplantation failed to decrease inflammation and to promote epithelial healing.

Conjunctival Surface Reconstruction

In primary pterygium, 4.2 per cent (2/47 eyes) were rated failure with recurrence. 17.0 per cent (8/47 eyes) were rated partial success or equal to grade 3 in our previous report[28] with some fibrovascular tissue invading the grafts but not into the cornea. The rest, 78.8 per cent (37/47 eyes) were rated success. Amniotic membrane was used to reconstruct the defects created by surgical removal of various conjunctival lesions in 17 cases (17 eyes) including nevus (7 eyes), conjunctival intraepithelial neoplasia or squamous cell carcinoma (5 eyes), papilloma (2 eyes) and squamous metaplasia resembling malignancy (3 eyes). 70.6 per cent or 12 eyes were rated as success with recovery of smooth, quiet and non-scarred surfaces. The rest (23.5%, 5 eyes) were rated as partial success with some fibrovascular tissue invading the graft, leading mild to moderate inflammation in the first couple of months but subsided with time. No case showed symblepharon or significant scarring and all patients were satisfied with the outcome.

In 7 cases with symblepharon, 5 were due to multiple surgeries for pterygium, and one was due to congenital lid abnormality. 28.6 per cent (2/7 eyes) of this subgroup were rated success. 28.6 per cent (2/7 eyes) had some recurrent symblepharon with lesser extent than the preoperative status. Vision was improved from finger counting to 20/40 in one case. Two cases received scleral grafts for scleral melt following pterygium excision combined with beta radiation and mitomycin 20 years and 7 years previously, respectively. One of them was rated failure because the amniotic membrane graft dissolved. We attributed it to severe ischemic bed of the sclera because subsequent conjunctival autograft also failed and dissolved in 2 weeks, and eventually the scleral graft also melted and dissolved. The other case was rated success as the epithelial defect healed in one week following AMT combined with conjunctival flap, presumably due to blood supply from the flap to the ischemic sclera.

Complications

We divided complications into 2 major groups (Table 5). Immediate complications, i.e., those developing within 1 month of surgery, included dellen formation (3 eyes) as a result of graft edema or graft loss, detachment of amniotic membrane (2 eyes), and conjunctivitis, lid abscess, and entropion (one eye). All 3 eyes with complications of dellen were successfully treated with frequent tear substitutes. Despite detachment of amniotic membrane, the epithelial defect healed rapidly in 1 week in the patient with bullous keratopathy and the patient became pain-free. The eye which deve-

loped conjunctivitis, lid abscess and entropion was from a patient with Steven Johnson syndrome as a result of bacterial infection and the membrane was detached and dissolved in that area. This complication was successfully treated with antibiotics and conjunctival flap to cover the bare sclera.

Late complications were those which developed after 1 month of surgery and the epithelial surface had healed completely. Three eyes developed corneal ulcer. The first case suffering Steven Johnson syndrome developed corneal ulcer at 1 month after surgery due to contamination of the eye drop. The second case with pseudophakic bullous keratopathy developed corneal ulcer at 2 months after surgery as a result of the dust in the eye. The third case, suffering from limbal deficiency and persistent corneal epithelial defect due to chemical burn, received autolimbal transplantation and amniotic membrane transplantation and penetrating keratoplasty 2 months later. These procedures resulted in a clear and stable corneal graft 3 months after penetrating keratoplasty, but developed bacterial corneal ulcer which was successfully treated with topical antibiotics. Three eyes developed minute corneal perforation in Steven Johnson syndrome at 2 months, 2 months and 9 months, respectively. In these three eyes, corneal perforation was less than 1 mm in diameter without signs of infection and was successfully treated with glue and contact lens.

CASE EXAMPLES

Case 1 For partial limbal deficiency

An HIV-positive 36 year-old male, diagnosed with Steven-Johnson syndrome and severe keratoconjunctivitis sicca, complained of painful irritation and inability to open his eyes. The vision was 20/200 in both eyes, and the conjunctiva was marked injected in both eyes and symblepharon was noted in the temporal conjunctiva of the left eye (Fig. 1A). Despite occlusion of both upper and lower puncta, he remained symptomatic with marked inflammation. AMT was performed in both eyes in two settings to cover the whole cornea and bulbar conjunctiva 5 millimeters from the limbus as a graft. After surgery, his vision improved to 20/80 and he felt much more comfortable, and there was no conjunctival injection in both eyes (Fig. 1B) at 3 month follow-up except at the previous symblepharon area in the left eye.

Case 2 For total limbal deficiency with combined allograft limbal transplantation

A 50 year-old female suffered from irritation and blurred vision for many years due to limbal deficiency from herpes simplex keratitis and severe keratoconjunctivitis sicca in her right eye, which had received penetrating keratoplasty 4 years ago. Visual acuity in her right eye was counting finger at 2 ft. Moderate conjunctival injection was noted in both eyes, and all four puncta were occluded for treating severe keratoconjunctivitis sicca. Conjunctivalization was noted 360° of her cornea (Fig. 1C). AMT was performed to cover the whole cornea and bulbar sclera 5-7 mm from the limbus in her right eye. After surgery, her eye had no irritation and the bulbar conjunctiva was smooth wettable without inflammation. Three months later, allograft limbal transplantation was performed resulting in improved vision of 20/50, clear cornea without vascularization, and quiet conjunctiva (Fig. 1D) after a follow-up of 18 months.

Case 3 For bullous keratopathy

A 66 year-old female suffered from irritation and tearing in her right eye due to corneal decompensation following extracapsular cataract extraction and anterior chamber lens implantation 5 years previously. Her vision was hand motion, and the cornea was edematous with epithelial bullae (Fig. 1E). Following epithelial scraping and AMT, the epithelial defect healed in 1 wk, and the transplanted amniotic membrane became transparent and difficult to be identified at 1 months (Fig. 1F). Eight months after surgery, her cornea remained edematous and bullae recurred, but she still enjoyed comfort without irritation for the entire follow-up of 18 months.

Case 4 For persistent epithelial defect

A one-eyed 87 year-old female developed a persistent corneal epithelial defect from herpes simplex keratitis for more than 2 weeks, which was refractory to treatment including bandage contact lens and frequent tear substitutes. Her vision dropped to 3/200, and the defect showed progressive thinning (Fig. 1G). AMT was performed to cover the epithelial defect, which showed healing at post-operative day 6 and completed in 3 weeks. At that time, amniotic mem-

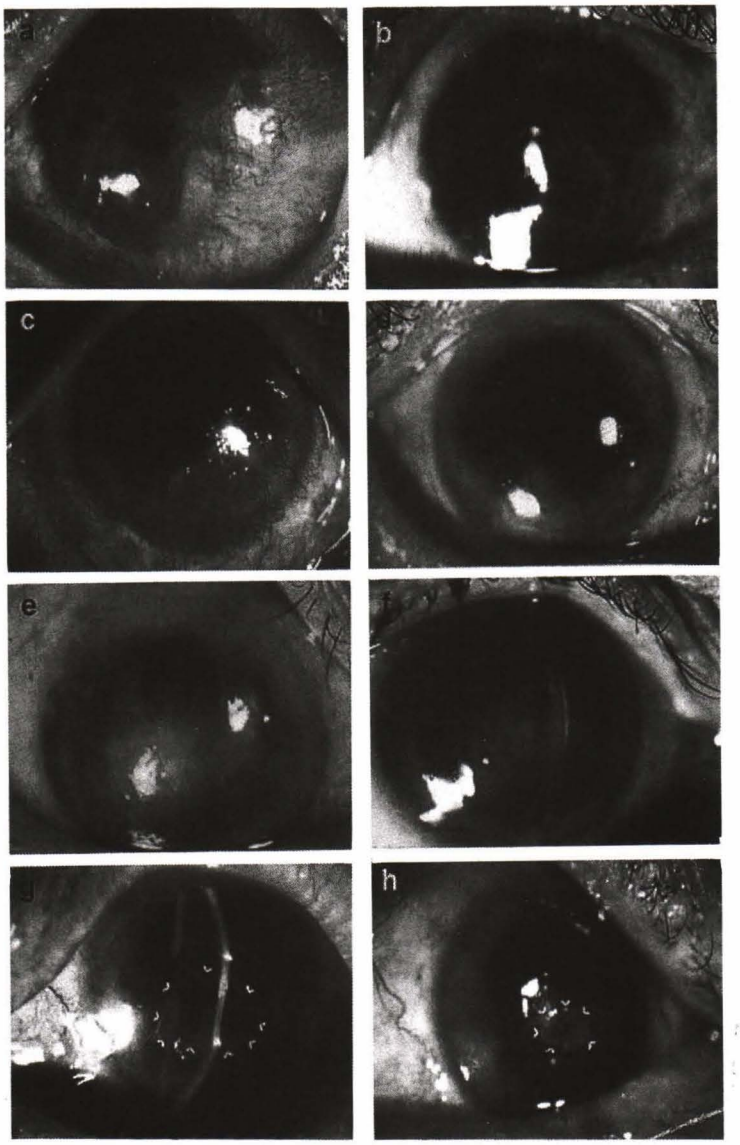

Fig. 1. Pre-operatively (left panel) and post-operative (right panel) appearance of corneal surface group, case 1 through 4. Pre-operative appearance of Steven Johnson's syndrome (Case 1) shows marked inflammation of the left eye (A). Post-operative AMT covers the whole cornea and bulbar conjunctiva 3 months in the left eye (B) resulting in a quiescent perilimbal conjunctiva. Case 2 limbal stem cell deficiency from HSV in the right eye, pre-operatively appearance (C) shows conjunctiva marked injected and vascularization 360° around the cornea. Post-operatively 12 months of amniotic membrane transplantation (AMT) and 9 months of limbal transplantation (D) resulted in quiescent perilimbal area (AMT area) and clear cornea. Case 3 had pseudophakic bullous keratopathy (E) in the right eye. Post-operatively AMT 1 month covered the cornea (F), the membrane is very transparent and smooth. Pre-operative appearance of case 4, persistent epithelial defect and thinning of the corneal stroma 5 mm at the central cornea (arrow) (G). Post-operatively epithelial defect has healed completely and stromal bed thickening at 3 weeks post-operation without fluorescein staining (H), amniotic membrane partially dissolved but still attached at the lesion (arrow).

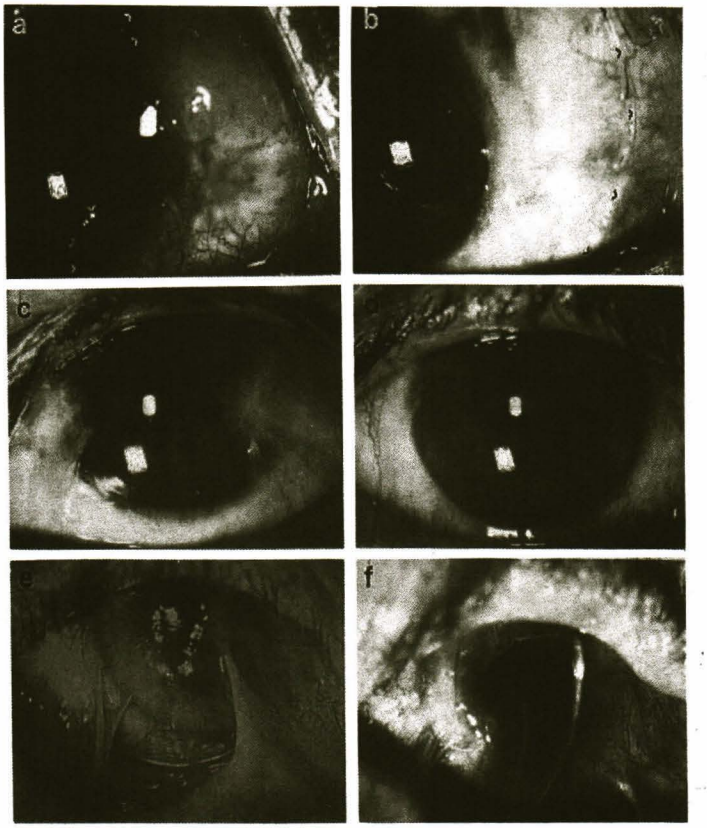

Fig. 2. Pre-operatively (left panel) and post-operative (right panel) appearance in conjunctival surface group of case 5 through 7. Case 5 has large CIN at the limbal area extending from 12 o'clock to 7 (arrow) (A), after tumor removal, amniotic membrane graft (arrow) 1 month is smooth and not injected (B). External appearance of case 6 showing bilateral heads of pterygium (C), and the appearance 1 month after amniotic membrane graft showing the graft is quiet, transparent and nonvascularized (D). The appearance of case 7 showing severe symblepharon (E) from multiple surgeries of pterygium excision, after lysis symblepharon cornea is clear but symblepharon recurred at 5 months (F).

brane partially dissloved but was still attached at the lesion (Fig. 1H) and totally dissolved at 2 months. Her vision improved to 20/50, the corneal stroma became thickened and the corneal epithelium did not show any recurrent breakdown after 8 months of follow-up.

Case 5 For removal of conjunctival tumor

A 42 year-old male had a large biopsy-proven conjunctival intraepithelial neoplasia (CIN) in his right eye. The lesion involved the limbus from 12 o'clock to 7 o'clock clockwise (Fig. 2A). The tumor was removed and an amniotic membrane graft of 20 mm in width was used to cover this large bare sclera. The graft was quiet without scarring at 1 month of follow-up (Fig. 2B) and stable up to 10 months of follow-up.

Case 6 For bilateral heads of primary pterygium

A 49 year-old female had a primary pterygium with nasal and temporal heads in her right eye invading onto the cornea (Fig. 2C). Pterygium excision and subconjunctival tissue removal for bilateral heads was performed. Amniotic membrane grafts were placed to cover both bare sclera

areas. The grafted area was quiet, wet, and without scar at 1 month (Fig. 2D) and no recurrence up to 10 months following surgery.

Case 7 For symblepharon lysis

A 69 year-old female complained of restriction of ocular motility and decreased vision in her right eye which had received more than 10 pterygium excisions (Fig. 2E). The visual acuity was counting finger at 2 ft, and severe symblepharon was noted in the inferior fornix, medial canthus and superior fornix. Her nasal eyelid was nearly attached to her cornea due to symblepharon. There was vascularization 360° around her cornea. Symblepharon was lysis 270° around the cornea and extended to both fornix. Amniotic membrane graft was placed to cover the whole cornea and conjunctival defect to the fornix. After surgery, her cornea was clear and smooth and the graft was also smooth, and not injected. Her visual acuity returned to 20/40. Unfortunately, some symblepharon recurred at medial and lateral of inferior fornix 5 months post-operation (Fig. 2F). Six months after surgery, the second lysis symblepharon was performed. Due to multiple recurrence of symblepharon, 0.02 per cent mitomycin was used during the operation to prevent fibrous tissue growth before amniotic graft was placed. The graft was quiet and pleased the patient until 3 months post-op when some recurrence happened again at the same site but did not invade the cornea.

DISCUSSION

In this study, we have shown that amniotic membrane is useful for both corneal and conjunctival surface reconstruction in difficult ocular surface problems (Table 3, 4). This technique has achieved success rates of 75.7 per cent, 80.6 per cent and 71.2 per cent and partial success rates of 17.9 per cent, 14.9 per cent and 20.6 per cent in total, corneal surface group, and conjunctival surface group, respectively. The usefulness and limitation of this procedure differs among different indications and are discussed below.

Limbal stem cell deficiency is a difficult disorder in which conventional penetrating keratoplasty has a limited role. Tseng et al[26] recently showed that amniotic membrane transplantation alone can be used to reconstruct corneal surfaces with partial limbal deficiency, i.e., there is part of the limbus retaining healthy limbal epithelial stem cells. In our series, when limbal deficiency was not severe, AMT alone could restore corneal surface smoothness and eliminate photophobia, case 1 for example, and improve the visual outcome. As the result shows, 6 eyes in the group of AMT alone had visual improvement at least 2 snellen line even though they still had corneal vascularization (Table 5). Furthermore, in this group, except 2 patients (7.4%), all others felt more comfortable, moist, less irritation, less keratinization and pleased with the result and asked to have the other eye done. The reason why the patients felt more moist in their eyes and less keratinization might be because the amniotic membrane can increase goblet cell density of conjunctiva[34]. Besides, cyclosporin is not required in AMT, this is beneficial for developing countries like Thailand where the high expense of cyclosporin is a serious problem. However, when limbal stem cell deficiency becomes total and diffuse, amniotic membrane transplantation alone is not sufficient[26] and requires additional limbal stem cell transplantation for corneal surface reconstruction[24-27]. Here we also support this concept as all 5 eyes receiving AMT combined with limbal allografts and 5 eyes receiving AMT combined with limbal autograft had visual improvement and symptomatic relief while 9 eyes receiving AMT alone could not improve the vision.

Bullous keratopathy, caused by endothelial dysfunction, generally requires penetrating keratoplasty to relieve the ocular discomfort and to improve vision. Lee et al[21] first reported that amniotic membrane can be used as an alternative to conjunctival graft to relief pain and recurrent erosion. We noted here amniotic membrane is an excellent alternative for countries like Thailand where there is shortage of corneal donors, to relieve pain and reduce surface inflammation while the patient is on the waiting list for corneal transplantation. Although the mechanism remains unclear, we are intrigued by the finding that symptomatic relief persists even when marked edema and recurrent bullae remain several months following AMT.

When there is a persistent epithelial defect, corneal ulcer and perforation can develop. The conventional methods to treat the defect include the use of contact lens, conjunctival flap[35, 36] or tarsorrhaphy[37]. Consistent with what has been reported by Lee et al[21] and Kruse et al [22], our result also noted an overwhelming suc-

cess of treating this indication. AMT can promote epithelial healing and prevent corneal perforation, and thus solve the surface healing problem refractory to medical treatments and wearing a contact lens. Furthermore, it also gave a good cosmetic result and improved vision (Case 4).

If the counjunctival defect is left unattended following the removal of a large lesion such as pterygium, CIN, symblepharon, tumor or scar, marked scarring may ensue. Frequently such a defect is too large to be covered by an autologous conjunctival tissue. Under such a circumstance, we noted that AMT is an excellent alternative. Similar to our previous report[28], AMT achieved 95.8 per cent success for primary pterygium. For removal of other conjunctival lesions, we noted 100 per cent success and partial success. In the success group, which was 78 per cent in pterygium excision and 70 per cent in the conjunctival lesion, the grafts were quiet, no scarring and had good cosmetic results. In the partial success group, the grafts became smaller because of slight fibrovascular tissue invasion. These tissues regressed and were difficult to notice after 5-6 months of follow-up. No patients had severe scarring, marked inflammation or symblepharon. However, in symblepharon, the result of amniotic graft was not satisfactory. This may be due to the fact that amniotic membrane acts as a substrate without living cells and cannot restore the host tissue that has altered epithelium and/or stroma prone for fibrovascular growth. Other adjunctive therapies directed to the latter abnormality may need to be incorporated in the future.

In this study, we did not note any sign of graft rejection, and no patient became clinically worse than before the surgery in terms of visual acuity or inflammation. Only one case developed mild entropion after symblepharon lysis, and this complication slowly resolved 2 months later without the need of surgical correction. Immediate complications were not serious and treatable. For example, bacterial conjunctivitis was successfully treated with topical antibiotics. The cause of infection was not directly linked to the preparation of amniotic membrane as microbial cultures of the amniotic membrane during preparation or following surgery were all negative. Late complications may or may not be related to the procedure itself as they can be derived from the underlying disorders. For example late occurrence of corneal ulcer and perforation developed after the surface had healed. Furthermore, it is well known that diseases such as bullous keratopathy, penetrating keratoplasty and Steven Johnson's syndrome have weak ocular surface defense and are prone to develop corneal ulcer. Among all ocular surface disorders in this study, it is worth noting that patients with Steven Johnson syndrome developed the most complications and showed the lowest success. Future studies to understand more about the action of amniotic membrane transplantation may lead to other clinical uses.

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REFERENCES

1. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. Am J Obstet Gynecol 1979; 134; 833-45.
2. Talmi TP, Finckelstein Y, Zohar Y. Use of human amniotic membrane as a biological dressing. Eur J Plast Surg 1990; 13: 160-2.
3. Ramakrishnan KM, Jayaraman V. Management of partial-thickness burn wounds by amniotic membrane: a cost-effective treatment in developing countries. Burns 1997; 23 (Suppl 1): S33-6.
4. Sharma SC, Bagree NM, Bhat AL, Banga BB, Singh MP. Amniotic membrane is an effective burn dressing material. Jpn J Surg 1985; 15: 140-3.
5. Bose B. Skin wound dressing with human amniotic membrane. Ann R Coll Surg Eng 1979; 61: 444-7.
6. Shun A, Ramsey-Stewart G. Human amnion in

the treatment of chronic ulceration of the legs. Med J Aust 1983; 2: 279-83.
7. Somerville PG. The possible use of amniotic membrane in chronic leg ulcers. Phlebologie 1982; 35: 223-9.
8. Silverton JS, Trelford JD, Roussere JT, Wolfe BM, Conti S. The use of amniotic membrane in acute massive full-thickness loss of the abdominal wall from clostridial myonecrosis. Ann Plast Surg 1979; 3: 558-66.
9. Subrahmanyam M. Amniotic membrane as a cover for microskin grafts. Br J Plast Surg 1995; 48: 477-8.
10. Waikakul S, Chunmniprasas K, Setasubun S, Vajaradul Y. Application of freeze-dried amniotic membrane: a control trial at the donor site of split thickness skin grafting. Bull Hosp Jt Dis Ortho Inst 1990; 50: 27-34.
11. Bleggi-Torres LF, Werner B, Piazza MJ. Ultrastructural study of the neovagina following the utilization of human amniotic membrane for treatment of congenital abscence of the vagina. Braz J Med Biol Res 1997; 3: 861-4.
12. Dhall K. Amnion graft for treatment of congenital abscence of the vagina. Br J Obstet Gynaecol 1984; 91: 279-82.
13. Zohar Y, Talmi YP, Finkelstein Y, Shvili Y, Sadov R, Laurian N. Use of human amniotic membrane in otolaryngologic practice. Laryngoscope 1987; 97: 978-80.
14. Yokomari K, Ohkubra M, Kitano Y, et al. Advantages and pitfalls of amnion inversion repair for the treatment of large unruptured omphalocele: results of 22 cases. J Pediatr Surg 1992; 27: 882-4.
15. Rennekampff HO, Dohrmann P, Fory R, Fandrich F. Evaluation of amniotic membrane as adhesion prophylaxis in a novel surgical gastroschisis model. J Invest Surg 1994; 7: 187-93.
16. Young RL, Cota J, Sund G, Mason BA, Wheeler JM. The use of an amniotic membrane graft to prevent post-operative adhesions. Fertil Steril 1991; 55: 624-8.
17. Trelford-Sauder M, Dawe EJ, Trelford JD. Use of allograft amniotic membrane for control of intra-abdominal adhesions. J Med 1978; 9: 273-84.
18. DeRoth A. Plastic repair of conjunctival defects with fetal membranes. Arch Ophthalmol 1940; 23: 522-5.
19. Panzardi D. Sul'uso delle membrane fetali come materiale per plastiche congiumtivali con paritcolare riguardo al loro impiego nello pterigio. Bulieltino D'Oculistica 1947; 26: 332-6.
20. Kim JC, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. Cornea 1995; 14: 473-84.
21. Lee S, Tseng SCG. Amniotic membrane trans-plantation for persistent epithelial defects with ulceration. Am J Ophthalmol 1997; 123: 303-12.
22. Kruse FE, Rohrschneider K, Volcker HE. Multilayer amniotic membrane transplantation for reconstruction of deep corneal ulcers. Ophthalmology 1999; 106: 1504-11.
23. Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. Br J Ophthalmol 1999; 83: 399-02.
24. Tsubota K, Satake Y, Ohyama M, et al. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Am J Ophthalmol 1996; 122: 38-52.
25. Shimazaki J, Hao-Yang Y, Tsubota K. Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. Ophthalmology 1997; 104: 2068-76.
26. Tseng SCG, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. Arch Ophthalmol 1998; 116: 431-41.
27. Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular surface disorders with corneal epithelial sem-cell transplantation. N Eng J Med 1999; 340: 1697-03.
28. Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. Ophthalmology 1997; 104: 974-85.
29. Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. Br J Ophthalmol 1998; 82: 235-40.
30. Tseng SCG, Prabhasawat P, Lee S. Amniotic membrane transplantation for conjunctival surface reconstruction. Arch Ophthalmol 1997; 124: 765-74.
31. Fujishima H, Shimazaki J, Shinozaki N, Tsubota K. Trabeculecomy with the use of amniotic membrane for uncontrollable glaucoma. Ophthalmic Surg Lasers 1998; 29: 428-31.
32. Choi YS, Kim JY, Wee WR, Lee JH. Effect of the application of human amniotic membrane on rabbit corneal wound healing after excimer laser photorefractive keratectomy. Cornea 1998; 17: 389-95.
33. Wang M, Gray T, Prabhasawat P, et al. Corneal haze is reduced by amniotic membrane matrix in excimer laser photoablation in rabbits. Invest Ophthalmol Vis Sci 1997; 38: S405.
34. Prabhasawat P, Tseng SCG. Impression cytology

study of epithelial phenotype of ocular surface reconstructed by preserved human amniotic membrane. Arch Ophthalmol 1997; 115: 1360-7.
35. Gundersen T. Conjunctival flaps in the treatment of corneal disease with reference to a new technique of application. Arch Ophthalmol 1958; 60: 880-8.
36. Insler MS, Pechous B. Conjunctival flaps revisited. Ophthalmic Surg 1987; 18: 455-8.
37. Welch C, Baum J. Tarsorrhaphy for corneal disease in patients with rheumatoid arthiritis. Ophthalmic Surg 1988; 19: 31-2.

การปลูกถ่ายเนื้อเยื่อรก รักษาโรคทางจักษุวิทยา†

ภิญนิตา ประภาสะวัต, พ.บ., ***, พนิดา โกสีย์รักษ์วงศ์, พ.บ.*, ***,*
วิภาวี บูรณพงศ์, พ.บ., ยงยุทธ วัชรดุลย์, พ.บ.**, ****

วัตถุประสงค์ : เพื่อศึกษาประสิทธิภาพของการรักษาโดยใช้เนื้อเยื่อรกรักษาโรคที่มีปัญหาทางด้าน ocular surface

วิธีการศึกษา : ได้ทำการผ่าตัดโดยใช้เนื้อเยื่อรกในผู้ป่วย 140 ตา (130 ราย) รักษาโรคของกระจกตาคือ limbal stem cell deficiency, bullous keratopathy, persistent epithelial defect, band keratopathy, prosthesis, กระจกตาอักเสบติดเชื้อและตาถูกสารเคมี และในกลุ่มโรคของเยื่อบุตาคือใช้เนื้อเยื่อรกเป็น graft หลังผ่าตัดลอกต้อเนื้อ, ผ่าตัดเนื้องอกของเยื่อบุตา, เลาะพังผืดที่เยื่อบุตาและคลุม scleral graft

ผลการรักษา : ผลการรักษาได้ผลดี 75.7% (106/140 ตา), ได้ผลดีบางส่วน 17.9% (25/140 ตา), และไม่ได้ผล 6.4% (9/140 ตา) ระยะการติดตามผลเฉลี่ย 6.6 เดือน (1-19 เดือน) ในกลุ่มโรคของกระจกตาและของเยื่อบุตา ผลการรักษาได้ผลดี 80.6% (52/73) และ 71.2% (54/67) ตามลำดับ, ได้ผลดีบางส่วน 14.9% (10/67) และ 20.6% (15/73) ตามลำดับ

สรุป : การผ่าตัดโดยใช้เนื้อเยื่อรกสามารถช่วยรักษาโรคที่มีปัญหาทางด้านของ ocular surface ได้ในหลายโรค สามารถช่วยเร่งการหายของแผล ลดการอักเสบและลดการเกิดแผลเป็นได้

คำสำคัญ : เนื้อเยื่อรก, เยื่อหุ้มรก, โรคของกระจกตา, โรคของเยื่อบุตา, ต้อเนื้อ, เนื้องอกของเยื่อบุตา

ภิญนิตา ประภาสะวัต, พนิดา โกสีย์รักษ์วงศ์,
วิภาวี บูรณพงศ์, ยงยุทธ วัชรดุลย์
จดหมายเหตุทางแพทย์ ฯ 2544; 84: 705-718

* ภาควิชาจักษุวิทยา,
** ภาควิชาศัลยศาสตร์ออร์โธปิดิคส์,
*** ศูนย์เนื้อเยื่อชีวภาพกรุงเทพ ฯ คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700
† สนับสนุนบางส่วนโดยทุนอุดหนุนการวิจัยของคณะแพทยศาสตร์ศิริราชพยาบาลประจำปี 2541 เคยเสนอในงานประชุม International Conference on Tussue Bank Asia Pacific Association of Surgical Tissue Banks ครั้งที่ 7 ที่เมืองกัวลาลัมเปอร์ ประเทศมาเลเซีย เดือนพฤศจิกายน 2541 งานประชุม Thai-Japan Joint Meeting ที่กรุงเทพ ฯ เดือนมกราคม 2542 และงานประชุม Annual Meeting of the Ocular Surface and Tear Conference ที่เมืองไมอามี รัฐฟลอริดา ประเทศสหรัฐอเมริกา เดือนพฤษภาคม 2542