Efficacy of Amniotic Membrane Patching for Acute Chemical and Thermal Ocular Burns

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Objective: *To study the efficacy of amniotic membrane patching (AMP) for acute chemical and thermal ocular burns and compare the results with a control group.*
Material and Method: *Fifteen patients (21 eyes) with acute ocular burn severity grading of II to IV were retrospectively reviewed. Thirteen eyes were treated with preserved AMP while eight eyes were treated with conventional treatment. Outcomes and complications were evaluated and compared between eyes in the AMP group and the control group with the same severity of burn.*
Results: *In the AMP group, the mean age was 36.9 ± 11.7 years (range, 20-58). The mean follow-up time was 8.0 ± 6.8 months (range, 1-20). Complete epithelialization was achieved in 69.2% (9/13 eyes) in total, 100% (5/5 eyes), 100% (3/3 eyes) and 20% (1/5 eyes) in grade II, III and IV respectively. Mean epithelial healing time in the AMP group was 10.4 ± 5.8 days (range, 4-20). Comparison of grade 2 and 3 burns showed that the AMP group in which patching was performed within 5 days resulted in faster epithelial healing, less corneal haze and limbal deficiency than in the group in which patching was performed after 5 days, and the control group (mean epithelial defect 7.0 ± 2.0, 19.5 ± 0.7, 9.9 ± 10.8 days respectively).*
Conclusion: *Adjunctive treatment of ocular burns with AMP promoted rapid epithelial healing and reduced corneal complication. Surgery performed in the early stage tended to yield a better outcome.*

Keywords: ***A****mniotic membranes, Amniotic membrane patching, Chemical burn, Thermal burn, Ocular burn*

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Acute burn is one of the most difficult ocular emergencies encountered by ophthalmologists. Chemical toxicity, thermal effect and the inflammatory process after the insults can produce extensive damage to the ocular surface and leads to limbal stem cell deficiency, persistent epithelial defect, corneal melting, perforation, neovascularization, and conjunctivalization[1]. Severe conjunctival burn can also extensively damage conjunctival epithelium, conjunctival stem cells, and goblet cells that results in a severe dry eye, keratinization, and symblepharon. Severity of the injury, treatment modality, and timing of treatment are factors that determine the prognosis of the injured eyes. Extensive and immediate eye irrigation remains the most important factor in reducing the severity of burn. The aims of the treatment are to promote the ocular surface healing by augmenting corneal epithelial growth and keratocyte production, to reduce the inflammation, prevent progressive tissue melting, minimize scarring, and restore vision as much as possible. Medical therapies, such as tear supplement, fibronectin, epidermal growth factor, topical and systemic ascorbate[2,3], collagenase inhibitor[1], citrate[3,4], tetracycline[5,6] and steroids[1,7,8] have been advised. Other interventions such as bandage contact lens[1], tenoplasty[9,10] and limbal stem cell transplantation[11] have also been proposed in the acute stage of burn to promote epithelial healing and improve the ocular surface conditions.

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Several reports[12-16] have addressed a new adjunctive treatment of acute ocular burn by using amniotic membrane transplantation (AMT). Most of these studies[12-14,16] report a favorable outcome with this treatment modality. The rational of the AMT is mainly its ability to promote epithelial healing and reduce the inflammation[13,17-19]. However, optimal time when AMP should be performed is still questionable. In the present study, the authors reviewed the efficacy of amniotic membrane patching (AMP) for acute ocular burn with different severity grading and compared it with the conventional method. The time between injury, AMP, and results of the treatment were recorded and analyzed.

Material and method

Patients

The present study was approved by the Committee for Protection of Human Subjects in Research at the Faculty of Medicine, Siriraj Hospital, Mahidol University. A retrospective chart-review was performed to study the efficacy of amniotic membrane patching (AMP) for the treatment of acute burns. From 1997-2003, 15 cases, 21 eyes of acute chemical and thermal burns with severity grading of 2 to 4 were identified. The patients were divided according to the treatment into 2 groups; group 1 (13 eyes) underwent AMP, and group 2 (8 eyes) was treated with conventional methods. For all eyes in the present study, the treatments comprised of eye irrigation, removal of remaining particle material, non preserved 1% methylprednisolone eye drop, prophylactic uses of topical 0.5% levofloxacin (cravit^{R}, Santen pharmaceutical, Osaka, Japan), non-preserved tear, autologous serum eye drop, 500 mg ascorbate orally three times a day and anti-glaucoma drugs when needed. AMP was performed for eyes in group 1 and a soft bandage contact lens was applied for group 2. All eyes were graded according to the Roper-Hall classification[20] of ocular burn.

The corneal epithelial defect size, epithelial healing time, results of treatment, and complications were recorded. Success was defined as complete epithelialization after AMP in group 1 or after conventional treatment in group 2. In cases that developed corneal neovascularization, impression cytology was performed to confirm the stage of limbal deficiency. For ocular burn grade II and III, results of the two treatment groups were then compared and analyzed.

Preparation of preserved human amniotic membrane

Amniotic membranes obtained from the Bangkok biomaterial center were used for all patients in the present study. Human placentas were obtained and prepared as previously described[19].

Surgical technique

In the AMP group, AMP was performed using the same technique for all eyes. Under a retrobulbar or subconjunctival anesthesia, amniotic membrane was removed from the storage media and transferred to the eye to cover the entire cornea and the whole ischemic area including limbus and conjunctiva. With basement membrane side turning up, the amniotic membrane was secured to the episclera and conjunctiva by interrupted 10-0 nylon sutures as shown in Fig 2 c. The amniotic membrane was removed when the epithelial defect was completely healed. In some cases in which the AM peeled off before complete epithelialization, AMP was repeated under the same technique. Postoperative medications included topical preservative free 1% methylprednisolone eye drop four times daily, topical 0.5% levofloxacin eye drop (cravit^{R}, Santen pharmaceutical, Osaka, Japan) four times daily, and other medications preoperatively used as previously mentioned.

Statistical analysis

Clinical data of AMP patients were described. The clinical results were presented in number and percent. Mean $\pm$ SD range were expressed for continuous variables. Clinical data were compared between AMP and control group. A p-value of less than 0.05 was considered significant difference.

Results

Fifteen patients (21 eyes) were divided into 2 groups, group 1 AMP group (13 eyes) and group 2 conventional treatment group (8 eyes). There were 14 males and 1 female with a mean age of 35.2 $\pm$ 11.4 years (range, 18-58). The mean age of the AMP group and conventional treatment group were 36.9 $\pm$ 11.7 years (range, 20-58) and 32.4 $\pm$ 10.9 years (range, 18-51 years) respectively. The mean follow-up time was 6.3 $\pm$ 5.6 months (range, 1-20), 8.0 $\pm$ 6.8 months (range, 1-20), 3.7 $\pm$ 2.6 months (range, 1-10) in control, for the AMP group and conventional treatment groups respectively. Demographic data, causes of burn are listed in Table 1 and Table 3.

In the AMP group, there were four eyes with severity grade II, four eyes with grade III, and five eyes with grade IV. The mean epithelial defect size in the AMP group was 92.7 $\pm$ 17.2% (range 40-100%). The

Table 1. Clinical data of AMP patients

Patient No.	Sex/Age yrs.	Agents/Eye	No. of AMP	Duration of each AMP month	Associated diseased		
					Glaucoma treatment	Cataract	Lid abnormal
1	M/58	Ak/OD	1	7	N	N	N
2	M/29	Ak/OD	1	4	N	N	N
2	M/29	Ak/OS	1	6	N	N	N
3	F/42	Th/OS	2	14, 7	Y; med	N	Entropion
4	M/49	Ak/OD	2	8, 11	Y; med	N	N
5	M/31	Ac/OD	1	10	N	N	N
5	M/31	Ac/OS	1	9	N	N	N
6	M/28	Ac/OS	1	6	N	N	Ectropion
7	M/35	Ak/OS	2	7, -	Y; med	N	N
2	M/58	Ak/OS	5	7, 20, 7, 21	Y; LDTC	Y	N
8	M/34	Ak/OD	1	12	N	N	N
9	M/20	Th/OD	2	21, 11	Y; med	N	N
10	M/36	Ac/OS	2	10, 10	Y; med	N	N

Ak = alkali, Ac = acid, Th = Thermal, AMP = amniotic membrane patching, Med = antiglaucoma medication, LDTC = Laser diode transcleral cyclophotocoagulation

mean duration between onset of injury and AMP was 5.1 ± 4.3 days (range, 1-14). The success rate (complete epithelialization) of the AMP group was 69.2% (9/13 eyes) in total, 100% (5/5 eyes), 100% (3/3 eyes) and 20% (1/5 eyes), for severity grade II, grade III and grade IV respectively. There was complete corneal epithelialization with improvement of visual acuity in all cases in grade II and grade III injuries (Fig. 1). In grade IV, due to the extensive ocular burn, complete epithelialization occurred only in one eye (Fig. 2). Among the success cases in the AMP group, complete epithelialization occurred in 10.4 ± 5.8 days (range, 4-20). In these eyes, AMP might be repeated more than once and the mean number of AMP performed for each eye was 1.7 ± 1.1 times (range, 1-5). The mean duration in which each amniotic membrane patching was attached to the ocular surface before peeling off was 9.8 ± 4.6 days (range, 4-21) and the mean follow up time was 8 ± 6.8 months (range, 1-20) as shown in Table 2.

Among the conventional treatment group in which no eye with severity grade IV was included, complete epithelialization was seen in 87.5% (7/8 eyes) with a mean healing of 9.9 ± 10.8 days (range, 4-33). The success rate of re-epithelialization was 100% (7/7 eyes) and 0% (0/1 eyes) in grade II and III respectively. The mean epithelial defect size was 71.9 ± 30.7%, and the follow up time was 3.7 ± 2.6 months.

Three eyes out of five with grade IV could not achieve complete epithelialization despite a repeated

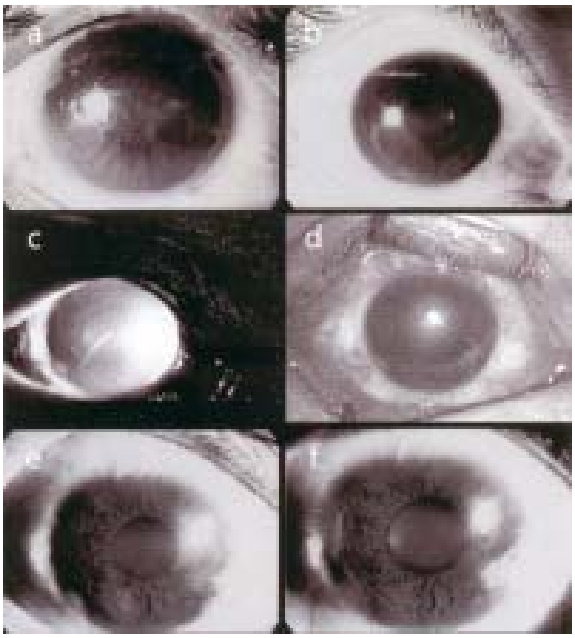

Fig. 1 (a) demonstrates ocular burn grade 3 pre-operative. (b) shows the same eye with clear cornea and non-inflamed conjunctiva seven months later. Another case with grade 3 burn pre-operative and 4 days post-operative are presented in (c) and (d) respectively. One month post-operative picture (e) demonstrates localized limbal deficiency proved by impression cytology which remains unchanged during 20 months of follow up (f)

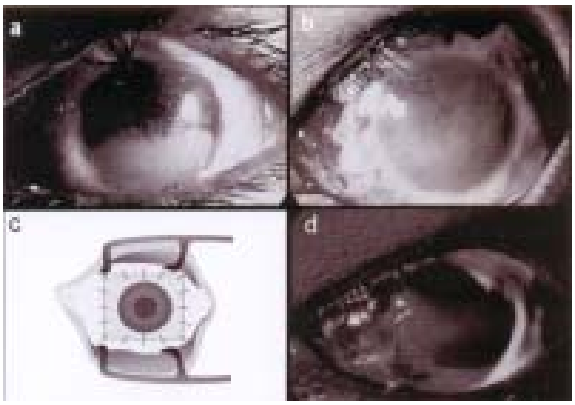

Fig. 2 Shows grade 4 ocular burn with large area of limbal ischemia and corneal haze (a). (b) illustrates AMP over the whole cornea and ischemic as drawn in diagram (c). Complete epithelialization of the same eye at day 13 (d)

AMP (2-5 times). Two out of these AMP failed eyes completely healed after tarsorrhaphy. One patient developed purulent discharge that grew coagulase negative staphylococci on the sixth day after AMP. The membrane was then removed and the infection was successfully treated with topical antibiotics (Table 2). At the end of the follow up, all failure eyes developed total conjunctivalization and decreased vision to hand motion.

Comparison of the AMP and conventional treatment group in acute burns

To compare the results of both treatment groups, eyes with the same severity from both groups were selected. However, in the conventional treatment group, there was no eye with severity grade IV. For this reason, only eyes with acute burn grade II and III were selected for comparison. In the AMP group, AMP performed within 5 days after the injury were referred to as "early AMP" and eyes AMP performed thereafter were "late AMP". For ocular burn grade II and III, successful treatment was achieved in all eyes in the AMP group while in 87.5% of the control (conventional) group. In the AMP group, complete epithelialization occurred more rapidly in the eyes with early AMP (7.0 $\pm$ 2.2 days) compared with those with late AMP (19.5 $\pm$ 0.7 days) and conventional treatment (9.9 $\pm$ 10.8 days). However, there was no statistical significance in re-epithelialization between eyes in the early AMP groups and control ($p = 0.0053$). It should be noticed that eyes with early AMP healed more rapidly than the other groups despite a larger area of defect and a more severe degree of burn. For the late AMP and conventional treatment, the time for epithelialization is longer in the former compared with the later (19.5 $\pm$ 0.7 and 9.9 $\pm$ 10.8). This may be due to a selection bias that occurred during the treatment. Eyes that were assigned to the conventional group tended to be less severe (7 cases with severity grade 2 and 1

Table 2. Clinical results of AMP patients

No.	Grade	Time between injury & surgery (days)	ED (%)	Time to re-epithelialization (days)	VA		Final result				FU (mo)
					pre	final	S/F	LD (Q)	Haze	Sym	
1	2	5	100	7	6/36	6/6	S	0	N	N	20
2	2	2	80	4	6/9	6/6	S	0	N	N	3.5
2	2	2	85	6	6/24	6/12	S	0	N	N	3.5
3	3	14	40	20	CF	CF	S	1	Y	N	16
4	3	7	100	19	6/36	6/12	S	1	N	N	4
5	3	3	100	10	6/36	6/6	S	0.5	N	N	5.5
5	3	3	100	9	6/36	6/36	S	0	N	N	5.5
6	3	5	100	6	6/60	6/12	S	1	N	N	1.5
7	4	1	100	infected	6/36	HM	F	3	Y	N	13
2	4	5	100	-	CF	HM	F	4	Y	N	20
8	4	4	100	13	6/60	6/36	S	0.5	N	N	1
9	4	1	100	-	HM	HM	F	4	Y	Y	6
10	4	14	100	-	6/36	HM	F	4	Y	Y	4
Mean		5.1$\pm$4.3	92.7$\pm$17.2	10.4$\pm$5.8							8.0$\pm$6.8

ED = epithelial defect, S = success, F = Failure, LD = limbal deficiency, Q = quadrant, Sym = symblepharon

case with grade 3) than those in the AMP group (4 cases with grade 2 and 4 cases with grade 3). The details of the comparison are shown in Table 3.

At the end of the follow up, all eyes that underwent early AMP demonstrated clear cornea without haze (Fig. 1a, b). Limited degree of limbal stem cell deficiency as demonstrated by localized conjunctivalization of the peripheral cornea developed in all cases (Fig. 1e) and was less in eyes with early AMP (Table 3). However, there was no progression of limbal deficiency during a long term follow up (Fig. 1f). Visual acuity improved in 62.5% (5/8) of eyes in the AMP group compared with 50% (4/8) of the conventional group. None of the eyes developed symblepharon.

Discussion

Ocular burn is one of the most devastating injuries that can lead to severe visual loss. Chemical and thermal effects of the injury along with subsequent inflammation usually result in corneal perforation, limbal deficiency, corneal neovascularization, corneal scaring, symblepharon, and severe dry eye. Among these conditions, persistent corneal epithelial defect is a tedious and challenging problem for ophthalmologists. This condition, if not properly treated, can lead to corneal melting, corneal perforation, secondary infection, extensive scarring, loss of vision, or even loss of eyes. Thus, one of the most important strategies of managing acute ocular burns is to promote rapid epithelialization.

The present study evaluated the clinical outcome of patients with moderate to severe acute ocular burns that were treated with amniotic membrane patching. The present results showed that AMP could be considered an adjunctive surgical procedure to promote epithelialization and prevent scaring especially for grade II and III burn compared with conventional treatment. Eyes with the same grade of burns healed more rapidly with minimal or no corneal haze in the AMP group. The present results are consistent with several investigators such as Kobayashi[14], Meller[12] and Tamhane[16].

The benefit of AMP in the treatment of acute ocular burns has been previously reported[12,14,16,21]. It promotes re-epithelialization by acting as a biological bandage lens that prevents mechanical trauma from the lids and corneal exposure, and reduces surface

Table 3. Clinical data compare between AMP and control group in grade II-III

	Control	AMP		
No. of eyes	8	8		
Mean age (yrs)	32.4 ± 10.9	37.1 ± 11.3		
Sex : Male (%)	100	89		
Agent: Ac/Alk/Th	3/2/3	3/4/1		
Grading 2/3	7/1	4/4		
Time between injury to admission (day)		Time between injury to AMP		
		≤ 5 days	> 5 days	Total
< 1 day	5	-	-	-
1-5 days	1	6	1	7
> 5 days	2	0	1	1
ED (%)	71.9 ± 30.7	94.2 ± 9.2	70.0 ± 42.4	88.1 ± 21.0
ED healing time (days)	9.9 ± 10.8	7.0 ± 2.2	19.5 ± 0.7	10.1 ± 6
Corneal conditions post Rx				
LD (quadrant)	0.7 ± 0.7	0.25 ± 0.4	3.0 ± 0	0.4 ± 0.5
Haze (%)	4/8 (50)	0/6 (0)	1/2 (50)	1/8 (12.5)
SPK (%)	4/8 (50)	0/6 (0)	0/2 (0)	0/8 (0)
Symblepharon (%)	0/8 (0)	0/6 (0)	0/2 (0)	0/8 (0)
VA improve at D/C	4/8 (50%)	3/6 (50%)	2/2 (100%)	5/8 (62.5)
VA improve at final F/U	7/8 (87.5)	5/6 (83.3)	2/2 (100%)	6/8 (75%)
FU (Mo)	3.7 ± 2.6	6.1 ± 6.7	10.0 ± 8.5	7.4 ± 6.7
Success	7/8 (87.5)	6/6 (100)	2/2 (100)	8/8 (100)

Ac = acid, Alk= alkali, Th = thermal, ED = epithelial defect, LD = limbal deficiency, SPK = superficial punctate keratitis, AMP = amniotic membrane patching

dryness. The AM also contains growth factors[22] which can promote proliferation and differentiation of epithelial cells, promote limbal progenitor cell[23,24] and maintain normal characteristic of human keratocytes[25]. It possesses a direct anti-inflammatory action, for example; several protease inhibitors[13,26] and anti-inflammatory proteins[27]. It can down regulate inflammatory cytokines such as interleukin 1[28]and 8 expression by keratocytes[29]. The stromal matrix of AM is also capable of excluding inflammatory cells (Park et al) from infiltrating the cornea[30]. These mechanisms may help augment epithelial healing, preserve the remaining limbal stem cells from further damage and allow them to grow underneath the membrane. Moreover, AM can suppress TGF-β[31,32] and prevent corneal and limbal fibroblast differentiation which may reduce corneal scarring and symblepharon formation. Taken together, these may explain a higher success rate of corneal epithelialization and a better ocular surface condition in the AMP group compared with the conventional group of the same severity. However, the authors realize that the present report is a non-randomized retrospective study with a selective bias regarding the treatment modality. Eyes that looked more severe (grade III) were more likely to have AMP (Table 3) and no eye with grade IV burns was assigned to the conventional group. However, even with the selective bias, eyes in the AMP group still revealed a better outcome compared with those in the conventional group, which was consistent with the study of Tamhane et al[16].

Various surgical techniques have been applied for AM transplantation in acute ocular burns[12,14,15,21]. The AM can be used as a graft, that is, for epithelialization to take place on its basement membrane side. In this case, the basement of AM may be used as a substitute for a damaged corneal or conjunctival basement membrane. When used as a patch as in the present study, epithelialization will take place underneath the AM. Meller et al[12] used both the patch and graft technique to treat acute ocular burns with favorable outcome. In the present study, the authors performed AMP with a different technique from Mellers'. Instead of covering the whole ocular surface from the upper lid margin to the lower one, the authors placed the AM stromal side up to cover the ischemic conjunctival area, limbal region and the whole cornea. The AM was sutured to the episcleral tissue. The authors feel that this technique is less time consuming, easier to perform and requires less amniotic membranes[33].

Optimum time to perform AMP is another important factor that can lead to a satisfactory outcome. The present study suggested that AMP performed within the first 5 days after the insults (early AMP) tended to yield a better result. This can be explained by the two waves of leukocyte infiltrations after burns that first occur within 24 hours and secondly start around day 7. The first wave is important for the recruitment of the second. It is known that chronic inflammation not only prevents re-epithelialization of the ocular surface, but is also a major factor leading to limbal stem cell deficiency in humans[34]. Therefore, AMP performed at the early stage may suppress and shorten the inflammation by several mechanisms mentioned above thus resulting in a better outcome.

In severe burns (stage IV), however, the authors as well as Joseph[15], Meller[12] and Tamhane[16] could not demonstrate adequate beneficial effect of AMP over conventional treatment. Amniotic membrane alone may reduce the inflammation and restore the conjunctival surface to some extent but cannot adequately prevent or improve limbal stem cell deficiency, which requires additional stem cell transplantation.

In summary, the present study suggests that AMP performed in the early stage of acute ocular burns is effective in promoting re-epithelialization and reducing corneal haze for mild to moderate burns (stage II and III). However, the authors realize that the present study is a retrospective, non-randomized study with a small number of cases. According to the present study, the authors suggest that early AMP can be considered as an effective treatment for acute ocular burns.

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ประสิทธิภาพของเยื่อถุงน้ำคร่ำในการรักษาภาวะบาดเจ็บจากสารเคมีและความร้อนที่ตา

ภิญนิตา ประภาสะวัต, ณัฐพร เทศะวิบุล, นวลจิรา ประกายรุ้งทอง, วิภาวี บูรณพงศ์

วัตถุประสงค์*: เพื่อศึกษาประสิทธิภาพการรักษาแผลกระจกตาด้วยเยื่อถุงน้ำคร่ำในผู้ป่วยที่ได้รับอันตรายจากสารเคมีและความร้อนที่ลูกตา โดยเปรียบเทียบกับกลุ่มที่ได้รับการรักษาแบบดั้งเดิม*

วัสดุและวิธีการ*: ศึกษาแบบย้อนหลังในผู้ป่วย 15 ราย (21 ตา) ที่ได้รับอันตรายจากสารเคมีหรือจากความร้อนที่ตาในความรุนแรงระดับ 2 ถึง 4 ผู้ป่วย 13 ตา ได้รับการรักษาโดยใช้เยื่อถุงน้ำคร่ำที่เก็บแช่แข็งเย็บคลุมกระจกตาและเยื่อบุตา และ 8 ตาได้รับการรักษาแบบดั้งเดิม ศึกษาและเปรียบเทียบผลการรักษาและผลแทรกซ้อนของทั้ง 2 กลุ่ม ในกลุ่มความรุนแรง 2 และ 3*

ผลการศึกษา*: ผู้ป่วยในกลุ่มใช้เยื่อถุงน้ำคร่ำ มีอายุเฉลี่ย 36.9 $\pm$ 11.7 ปี (20-58 ปี) ระยะการติดตามเฉลี่ย 8.0 $\pm$ 6.8 เดือน (1-20 เดือน) แผลที่กระจกตาหายดี 69.2% (9/13 ตา) จากทั้งหมดและ 100% (5/5 ตา), 100% (3/3 ตา) และ 20% (1/5 ตา) ในกลุ่มความรุนแรงที่ 2, 3 และ 4 ตามลำดับ ระยะเวลาโดยเฉลี่ยที่แผลกระจกตาหาย 10.4 $\pm$ 5.8 วัน (4-20 วัน) เมื่อเปรียบเทียบตาในระดับความรุนแรง 2 และ 3 พบว่ากลุ่มที่ทำผ่าตัดใช้เยื่อถุงน้ำคร่ำภายในเวลา 5 วันหลังเกิดอุบัติเหตุ จะมีการหายของแผลเร็วกว่า, มีกระจกตาขุ่นและภาวะ limbal deficiency น้อยกว่า กลุ่มที่ทำผ่าตัดช้าเกิน 5 วัน และกลุ่มที่รักษาแบบดั้งเดิม (ระยะเวลาแผลหายเฉลี่ย 7.0 $\pm$ 2.0, 19.5 $\pm$ 0.7, 9.9 $\pm$ 10.8 วัน ตามลำดับ*

สรุป*: การใช้เยื่อถุงน้ำคร่ำช่วยในการรักษาตาที่ได้รับอันตรายจากสารเคมีหรือความร้อนสามารถช่วยทำให้แผลกระจกตาหายเร็วและกระจกตามีสภาพดีขึ้น การทำผ่าตัดเร็วจะช่วยให้ได้ผลที่ดีกว่า*