·Clinical Research·

Amniotic membrane covering promotes healing of cornea epithelium and improves visual acuity after debridement for fungal keratitis

Bo Zeng [1], Ping Wang [2], Ling-Juan Xu [3], Xin-Yu Li [3], Hong Zhang [3], Gui-Gang Li [3]

[1]Department of Ophthalmology, Wuhan General Hospital of Guangzhou Military, Wuhan 430070, Hubei Province, China
[2]Department of Ophthalmology, Renhe Hospital of China Three Gorges University, Yichang 443001, Hubei Province, China
[3]Department of Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Co-first authors: Bo Zeng and Ping Wang
Correspondence to: Gui-Gang Li. Department of Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Dadao, Wuhan 430030, Hubei Province, China. guigli@163.com
Received: 2013-12-04 Accepted: 2014-06-23

Abstract

• AIM: To investigate the effect of amniotic membrane covering (AMC) on the healing of cornea epithelium and visual acuity for fungal keratitis after debridement.

• METHODS: Twenty fungal keratitis patients were divided into two groups randomly, the AMC group and the control group, ten patients each group. Both debridement of the infected cornea tissue and standard anti-fungus drugs treatments were given to every patients, monolayer amniotic membrane were sutured to the surface of the entire cornea and bulbar conjunctiva with 10-0 nylon suture for patients in the AMC group. The diameter of the ulcer was determined with slit lamp microscope and the depth of the infiltration was determined with anterior segment optical coherence tomography. Uncorrected visual acuity (UCVA) was tested before surgery and three month after healing of the epithelial layer. The healing time of the cornea epithelium, visual acuity (VA) was compared between the two groups using *t*-test.

• RESULTS: There was no statistical difference of the diameter of the ulcer, depth of the infiltration, height of the hypopyon and VA between the two groups before surgery (P>0.05). The average healing time of the AMC group was 6.89±2.98d, which was statistically shorter than that of the control group (10.23±2.78d) (P<0.05). The average UCVA of the AMC group was 0.138±0.083, which was statistically better than that of the control group (0.053±0.068) (P<0.05).

• CONCLUSION: AMC surgery could promote healing of cornea epithelium after debridement for fungal keratitis and lead to better VA outcome.

• KEYWORDS: amniotic membrane; fungal keratitis; cornea epithelium; visual acuity
DOI:10.3980/j.issn.2222-3959.2014.05.08

Zeng B, Wang P, Xu LJ, Li XY, Zhang H, Li GG. Amniotic membrane covering promotes healing of cornea epithelium and improves visual acuity after debridement for fungal keratitis. *Int J Ophthalmol* 2014;7(5):785-789

INTRODUCTION

Fungal keratitis is a kind of serious cornea infection that could eventually lead to blindness or loss of the eyeball if not treated effectively. Owing to a great variety of fungal pathogens, complex clinical manifestations, and limited antifungal medications, fungal keratitis is often difficult to treat, thus most of the patients need surgery treatment [1]. Debridement of the infected cornea tissue has been proved to be effective in removing the fungal pathogens and help to control the infection, unfortunately, delayed healing of the cornea epithelium may lead to over scarring of the cornea stroma that could impair the visual acuity (VA) of the patients [2]. Amniotic membrane (AM) has been widely used to promote the healing of epithelial layer of cornea and conjunctiva for none-infectious ulcer for a long time [3-9], but only a few surgeons reported the influence of AM for infected cornea ulcer[10-15]. We tested the influence of amniotic membrane covering (AMC) on the healing of cornea epithelium and VA restoration for fungal keratitis after debridement in this study.

SUBJECTS AND METHODS

Patients This study was approved by the ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, informed consent was obtained from each patient in accordance with the Declaration of Helsinki. The medical charts of twenty patients who underwent debridement with or without AMC for fungal keratitis by one doctor (Li GG) from 2012 to 2013 were reviewed. The diagnosis of fungal keratitis was based on the result of positive fungal hyphae corneal scraping under microscope with or without a positive fungal culture. A positive fungal culture was defined by a confirmatory growth of fungal colonies on Columbia blood medium plates. The patients were divided into two groups, the AMC group and the control group, ten patients in each group.

Antifungal Treatment Antifungal agents, 5% Natamycin (Alcon, USA), 0.25% Amphotericin B (Huabei Pharmaceutical Group, China) and 0.2% Fluconazole (Pfizer Inc. USA) were administered topically, as eye drops every two hours. Fluconazole injection was also used systemically, intravenous 0.2 g every day. Antifungal therapy was administered continuously before and after surgery. The frequency of antifungal therapy was maintained or tapered according to the clinical situation. At admission, patients were initially treated with topical broad-spectrum antibiotics in prevision of bacterial infection.

Amniotic Membrane Covering Procedure Human AM was prepared and preserved as previously reported [4,5]. Informed consents were obtained from all the patients prior to the surgery. Under peribulbar anesthesia, the necrotic tissue at the base of the ulcer was debrided and sent for cultures. The rolled-up edge of the ulcer or the loosely adhered epithelium adjacent to the ulcer was also removed. AM that could cover the whole cornea and bulbar conjunctiva was placed with its epithelial side up and secured with running 10-0 nylon sutures around the limbus and bulbar conjunctiva. At the end of the surgery, Chlortetracycline eye ointment (Beijing Shuangji Pharmaceutical Co., Ltd. China) was applied, and the eye was patched for 3h, then administration of the antifungal eye drops was resumed.

Main Outcome Measure The main outcome measure were 1) The morphology of the lesion (size, and depth of the infiltration, height of the hypopyon). The size of the lesion was recorded by the diameter, which is the average of the largest and smallest diameter of the defect of the epithelium. The depth of the infiltration was determined using anterior segment optical coherence tomography (As-OCT) examination and recorded as the ratio of infiltration to the whole thickness of the cornea. The height of the hypopyon in the anterior chamber was examined under slit microscope and recorded in millimeters; 2) Preoperative, postoperative uncorrected visual acuity (UCVA) were tested and recorded using international standard VA chart, (VA worse than 0.02 was recorded as 0), and the VA was tested before and three months after surgery; 3) Fungal pathogen in cornea scraping and culture; 4) Epithelia healing time in days, shown by cornea fluorescein staining; 5) Treatment failure necessitating second debridement or therapeutic penetrating keratoplasty (TPK).

Statistical Analysis All tests were performed at least in triplicate. Data sets were expressed as mean±SD and tested with Student's t-test between two sets of tests. A P-value of less than 0.05 was considered statistically significant.

RESULTS

As shown in Table1 and Table 2, there was no statistical difference between the average diameter and depth of the lesion, height of the hypopyon of two groups ($P>0.05$). Nine out of ten patients got cured of the fungal infection and one patient needed TPK in the AMC group, one patient underwent a second debridement. In contrast, eight out of ten patients got cured of the fungal infection, with two patients underwent a second debridement and two patients needed TPK in the control group.

Amniotic Membrane Covering Shortened the Healing Time of the Cornea Epithelium and Led to Better Visual Acuity As shown in Table 3, for the nine patients in the AMC group and eight patients in the control group that got total recovery of the cornea epithelium and removal of the fungal pathogen, the time needed for healing of the cornea epithelium was statistically shorter in the AMC group than that of the control group ($P<0.05$). The scarring and opacity of the cornea stroma in AMC group was less than that of the control group, which led to better uncorrected VA in the AMC group, with statistical difference ($P<0.05$). As shown in Figure 1, No.5 patient in the AMC group, with a 8 mm diameter defect of cornea epithelium (A), 0.3 cornea thickness depth of infiltration (D), underwent lesion debridement (D, red line) and AMC surgery, AM could be shown with OCT (B, E), cornea epithelium healed within ten days, with minor scar formation in the stroma (C, F). In contrast, Figure 2 demonstrated that No.13 patient in the control group, also with a 8 mm diameter defect of cornea epithelium (A), 0.3 cornea thickness depth of infiltration (D), underwent lesion debridement (D, red line) but not AMC

Table 1 Demographic data

Groups	Sex/Age (a)	Eye	Cornea scraping	Fungal culture	Needing second debrition	Needing TPK
AMC group						
1	M/62	OS	+	Fusarium +	N	N
2	F/63	OS	+	Fusarium +	N	N
3	M/47	OD	+	-	N	N
4	M/62	OS	+	Fusarium +	N	N
5	F/58	OS	+	Fusarium +	N	N
6	F/63	OS	+	Aspergillus +	Y	N
7	F/61	OS	+	-	N	N
8	M/47	OD	+	Aspergillus +	N	N
9	M/56	OS	+	Aspergillus +	N	Y
10	F/52	OD	+	Fusarium +	N	N
Control group						
11	M/47	OS	+	Fusarium +	N	N
12	M/48	OS	+	Fusarium +	N	N
13	M/60	OD	+	-	Y	N
14	M/50	OS	+	Fusarium +	N	N
15	M/46	OS	+	Fusarium +	N	N
16	M/67	OS	+	Aspergillus +	N	Y
17	F/50	OS	+	-	Y	N
18	M/72	OD	+	Fusarium +	N	N
19	M/56	OD	+	Aspergillus +	N	Y
20	M/44	OD	+	+	N	N

+: Positive; -: Negative; Y: Yes; N: Not.

Table 2 Diameter, depth of the infiltration of the cornea and height of hypopyon $\bar{x} \pm s$

Groups	Diameter (mm)	Depth	Hypopyon (mm)
AMC	6.65±1.25	0.41±0.12	1.3±1.42
Control	6.7±1.09	0.39±0.11	1.1±1.29
P	0.925	0.702	0.745

Table 3 Healing time of the cornea epithelium, uncorrected visual acuity before and after the surgery $\bar{x} \pm s$

Groups	Healing time (d)	VA-pre-op.	VA-post-op.
AMC	[1]6.89±2.98	0.024±0.031	0.138±0.083[a]
Control	10.23±2.78	0.046±0.063	0.053±0.068
P	0.018	0.337	0.038

pre-op.: Pre-operation; post-op.: Post-operation; [a] $P<0.05$.

surgery, without AM covering the surface of cornea, Natamycin adherd to the surface of stroma cornea (B, E) which interfered with the healing of cornea epithelium, thus he experienced the second time lesion debridement, epithelium healed twenty-one days later, with apparent scar formation in the stroma (C, F).

DISCUSSION

It has been well known that AM could promote the healing of cornea epithelium in none-infectious cornea ulcer [3-9]. Liu *et al* [3] reported that AM makes epithelial cell migration easier, and strengthen the basal cells connect. Boudreau *et al*[16] found that AM could prevent apoptosis of epithelial cells and accelerate corneal epithelium healing through providing natural polymer vector for corneal epithelial cells. Thomasen *et al*[17] confirmed that AM can produce some growth factors such as basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and transforming growth factor-β (TGF- β) that could promote the growth of corneal epithelial cells. Furthermore, transplanted AM has been proved to integrate with cornea stroma in corneal ulcers[18,19].

While for fear of the interference of the control of pathogens like fungus, bacteria and acanthamoeba, only a few authors reported the use of AM in infectious keratitis [10-15], Sangwan and Basu [20] found that AM has antimicrobial properties. Das *et al* [21] once even reported a case of fungal keratitis following AM transplantation for bullous keratopathy. As for fungal keratitis, Kim *et al* [10] reported that AM transplantation seems to be a useful adjunctive surgical

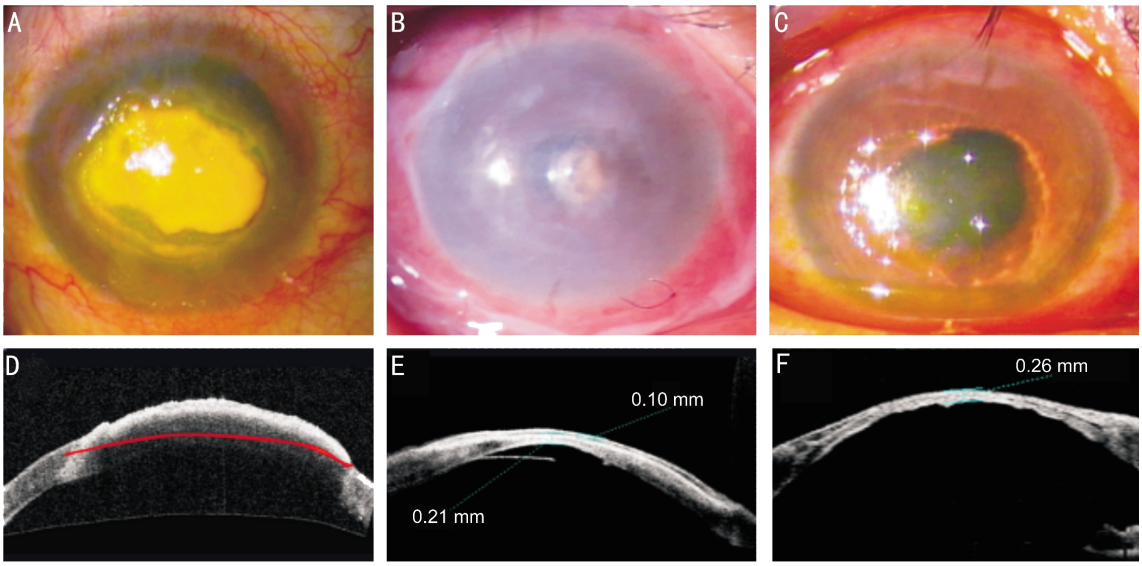

Figure 1 A case of the amniotic membrane covering group observed under slit lamp microscopy and As-OCT A, D: Before surgery; B, E: Two days after surgery, with AM covering the surface of cornea, the thickness of AM was approximately 100 μm as shown in the OCT figure E; C, F: Ten days after surgery, the AM had been removed, the cornea epithelium healed with scar formation in the stroma, uncorrected visual acuity 0.2.

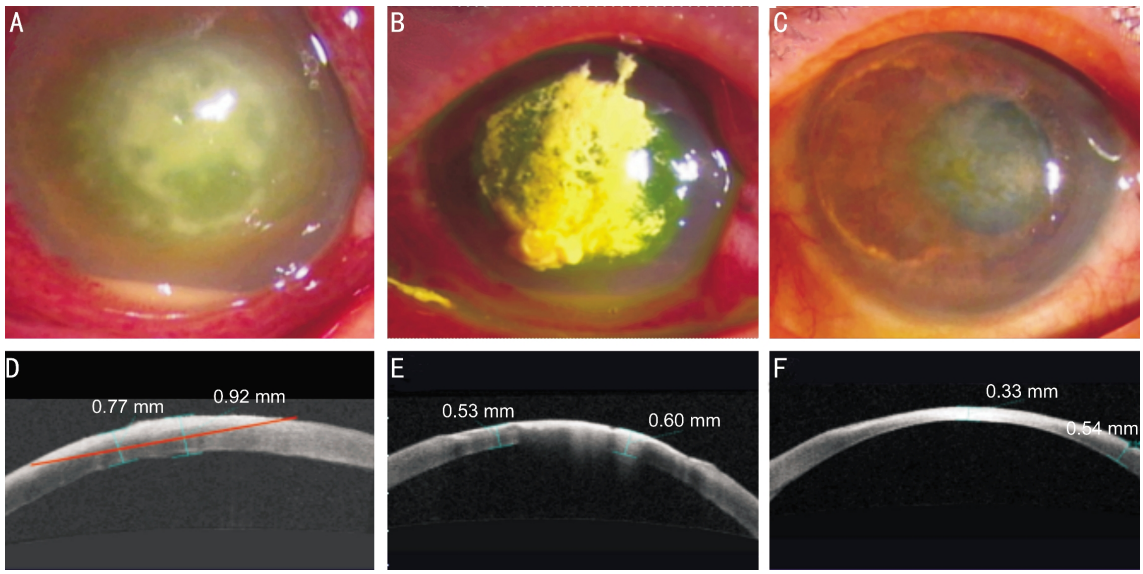

Figure 2 A case of the control group observed under slit lamp microscopy and As-OCT A, D: before surgery; B, E: two days after surgery, without AM covering the surface of cornea, but with Natamycin adhering to the surface of stroma; C, F: 21d after surgery, the cornea epithelium healed with scar formation in the stroma, uncorrected visual acuity 0.1.

procedure for the management of infectious corneal ulcer like staphylococcus species, pseudomonas species, acanthamoeba species, fungus, by promoting wound healing and reducing inflammation. Chen *et al* [11] reported that AM is effective in promoting epithelialization and preventing corneal perforations in acute fungal keratitis, and there is no risk of rejection. However, the risk of persistent or recurrent infection necessitates continued antifungal treatment and patient monitoring. Both of these two papers emphasized the importance of removal of the pathogen and medical treatment for success of infection control and epithelial healing.

In this study, using randomized prospective controlled sequence method, we observed the influence of AMC surgery on the outcome of fungal keratitis treatment after debridement. Because there was no statistical difference of the lesion before treatment, more cases got infection cured in the AMC group than that in the control group (nine *vs* eight), faster epithelium healing and better VA in the AMC group, thus we conclude that AMC could promote the cornea epithelium healing and improve the VA outcome for fungal keratitis patients. The reduction of inflammation during the healing of the epithelium by AM reduced the scarring of the stroma that account for better VA after surgery [22]. Our experience also indicating that thorough removal of the

fungal pathogen with debridement surgery before AMC and effective antifungal drugs are also necessary for the cure of infection and rebuilding of visual function.

ACKNOWLEDGEMENTS

Foundations: Supported by National Natural Science Foundation of China (No.81200661); Nature Science Foundation of Hubei Province (No.2010CDB09802); Wuhan Chenguang Plan Grant (No.201150431124)

Conflicts of Interest: Zeng B, None; **Wang P,** None; **Xu LJ,** None; **Li XY,** None; **Zhang H,** None; **Li GG,** None.

REFERENCES

1 Dursun D, Fernandez V, Miller D, Alfonso EC. Advanced fusarium keratitis progressing to endophthalmitis. *Cornea* 2003;22(4):300-303

2 Sun GH, Li SX, Gao H, Zhang WB, Zhang MA, Shi WY. Clinical observation of removal of the necrotic corneal tissue combined with conjunctival flap covering surgery under the guidance of the AS-OCT in treatment of fungal keratitis. *Int J Ophthalmol* 2012;5(1):88-91

3 Liu J, Sheha H, Fu Y, Liang L, Tseng SC. Update on amniotic membrane transplantation. *Expert Rev Ophthalmol* 2010;5(5):645-661

4 Lee S, Tseng SCG. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol* 1997;123(3):303-312

5 Chen HJ, Pires RTF, Tseng SCG. Amniotic membrane transplantation for severe neurotrophic corneal ulcers. *Br J Ophthalmol* 2000;84(8):826-833

6 Grau AE, Durán JA. Treatment of a large corneal perforation with a multilayer of amniotic membrane and TachoSil. *Cornea* 2012;31(1):98-100

7 Dietrich T, Sauer R, Hofmann-Rummelt C, Langenbucher A, Seitz B. Simultaneous amniotic membrane transplantation in emergency penetrating keratoplasty: a therapeutic option for severe corneal ulcerations and melting disorders. *Br J Ophthalmol* 2011;95(7):1034-1035

8 Rauz S, Saw VP. Serum eye drops, amniotic membrane and limbal epithelial stem cells--tools in the treatment of ocular surface disease. *Cell Tissue Bank* 2010;11(1):13-27

9 Mohan S, Budhiraja I, Saxena A, Khan P, Sachan SK. Role of multilayered amniotic membrane transplantation for the treatment of resistant corneal ulcers in North India. *Int Ophthalmol* 2014; 34 (3): 485-491

10 Kim JS, Kim JC, Hahn TW, Park WC. Amniotic membrane transplantation in infectious corneal ulcer. *Cornea* 2001;20(7):720-726

11 Chen HC, Tan HY, Hsiao CH, Huang SC, Lin KK, Ma DH. Amniotic membrane transplantation for persistent corneal ulcers and perforations in acute fungal keratitis. *Cornea* 2006;25(5):564-572

12 Mattila JS, Korsbäck A, Krootila K, Holopainen JM. Treatment of Pseudomonas aeruginosa keratitis with combined corneal cross-linking and human amniotic membrane transplantation. *Acta Ophthalmol* 2013;91(5): e410-e411

13 Kheirkhah A, Tabatabaei A, Zavareh MK, Khodabandeh A, Mohammadpour M, Raju VK. A controlled study of amniotic membrane transplantation for acute Pseudomonas keratitis. *Can J Ophthalmol* 2012; 47(3):305-311

14 Barequet IS, Habot-Wilner Z, Keller N, Smollan G, Ziv H, Belkin M, Rosner M. Effect of amniotic membrane transplantation on the healing of bacterial keratitis. *Invest Ophthalmol Vis Sci* 2008;49(1):163-167

15 Thatte S, Gupta L.Amniotic membrane transplantation in surgically induced necrotizing scleritis with peripheral ulcerative keratitis. *Middle East Afr J Ophthalmol* 2012;19(4):419-421

16 Boudreau N, Sympson CJ, Werb Z, Bissell MJ. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. *Science* 1995;267(5199):891-893

17 Thomasen H, Pauklin M, Steuhl KP, Meller D. Comparison of cryopreserved and air-dried human amniotic membrane for ophthalmologic applications. *Graefes Arch Clin Exp Ophthalmol* 2009;247(12):1691-1700

18 Gatzioufas Z, Sauter M, Hasenfus A, Smola S, Seitz B. In vivo analysis of stromal integration of multilayer amniotic membrane transplantation in corneal ulcers. *Am J Ophthalmol* 2012;153(2):379

19 Nubile M, Dua HS, Lanzini M, Ciancaglini M, Calienno R, Said DG, Pocobelli A, Mastropasqua R, Carpineto P. *In vivo* analysis of stromal integration of multilayer amniotic membrane transplantation in corneal ulcers. *Am J Ophthalmol* 2011;151(5):809-822

20 Sangwan VS, Basu S. Antimicrobial properties of amniotic membrane. *Br J Ophthalmol* 2011;95(1):1-2

21 Das S, Ramamurthy B, Sangwan VS. Fungal keratitis following amniotic membrane transplantation. *Int Ophthalmol* 2009;29(1):49-51

22 Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea* 2000;19(3):348-352