

# Transplantation of preserved human amniotic membrane for perforated bacterial corneal ulcer and persistent epithelial defects: Cases Study

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## ABSTRACT

Suhardjo, SG Indrawati, and Agus S - *Transplantation of preserved human amniotic membrane for perforated bacterial corneal ulcer and persistent epithelial defects, Cases Study.*

**Background:** Human amniotic membrane has anti-adhesive properties and is felt to promote epithelization and decrease inflammation. Fresh human amniotic membrane transplantation has been shown to be effective in the reconstruction of the corneal surface in the setting of persistent epithelial defects, sterile corneal ulcerations, and partial limbal stem cell deficiency.

**Objectives:** The purpose of this study is to report two cases of perforated bacterial corneal ulcer and one case persistent epithelial defect treated by preserved human amniotic membrane transplantation and to determine whether preserved human amniotic membrane transplantation can be used as an alternative method for treating perforated bacterial corneal ulcer.

**Methods:** Observed human amniotic membrane transplantation was performed on 2 eyes of 2 patients with perforated bacterial corneal ulcer and one case with persistent epithelial defect of different causes. The amniotic membrane was placed on the surface of the cornea in overlay and secured by interrupted 10.0 nylon sutures to the surrounding conjunctiva.

**Results:** There was significant improvement of the visual acuity in case 3 with persistent epithelial defect. Despite absence of improvement of the visual acuity in cases 1 and 2, the epithelization occurred in all patients and the perforated ulcers healed. As a wound dressing, human amniotic membrane has a number of benefits: in both patients the decreased inflammatory reaction, diminishes pain and increases the ocular mobility, and may help in prediction of readiness for grafting.

**Conclusion:** Preserved human amniotic membrane transplantation may be considered as an alternative method for treating perforated bacterial corneal ulcer which is refractory to conventional treatment.

**Key words:** amniotic membrane transplantation - decrease inflammation - perforated corneal ulcer - persistent epithelial defect - readiness for grafting.

## ABSTRAK

Suhardjo, SG Indrawati, dan Agus Supartoto- *Pencangkokan membran emnion awetan pada ulkus kornea bakteri perforasi dan defek epitel kornea menetap: Penelitian pada tiga kasus.*

**Latar belakang:** Membran amnion manusia memiliki sifat anti adhesi dan diperkirakan mampu memacu epitelisasi serta mengurangi reaksi radang. Pencangkokan membran amnion manusia segar telah menunjukkan keberhasilan pada defek epitelial kornea yang menetap, ulkus kornea steril, dan defisiensi sel stem limbal partial.

**Tujuan:** Tulisan ini bertujuan untuk melaporkan dua kasus ulkus kornea bakterial yang telah mengalami perforasi dan satu kasus defek epitel kornea menetap yang diterapi dengan transplantasi membran amnion awetan dan untuk mengetahui apakah transplantasi membran amnion manusia yang telah diawetkan dapat dipakai sebagai metode alternatif untuk penatalaksanaan ulkus kornea bakterial yang telah mengalami perforasi.

**Bahan dan cara:** Transplantasi membran amnion awetan dilakukan pada 2 mata dari 2 penderita ulkus kornea bakterial yang telah mengalami perforasi dan satu kasus defek epitel kornea persisten dengan penyebab yang berbeda. Pencangkokan dilakukan dengan teknik overlay, dijahit dengan benang IO.0 pada konjungtiva.

**Hasil:** Pada kasus ketiga terdapat perbaikan tajam penglihatan yang bermakna. Meskipun tidak didapatkan peningkatan ketajaman penglihatan pada kasus 1 dan 2, pada kedua penderita terjadi epitelisasi dan penutupan ulkus. Sebagai penutup luka membran amnion memiliki beberapa keunggulan : pada ketiga penderita terjadi penurunan reaksi inflamasi dan rasa sakit, peningkatan mobilitas bola mata, dan membantu dalam kemungkinan dilakukannya transplantasi.

**Simpulan:** Transplantasi membran amnion manusia awetan merupakan metode alternatif untuk terapi ulkus kornea bakterial yang telah mengalami perforasi yang sukar disembuhkan dengan terapi konvensional.

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## INTRODUCTION

The management of infected corneal ulcers can be one of the most challenging problems in the clinician's practice. Although in some severe complicated cases the patients must be hospitalized, most patients with infected corneal ulcers can be well cared on an out patient basis by the clinician using equipment available in the office or local hospitals<sup>1</sup>.

Despite many medical and surgical approaches that have been developed to treat them, ulcers of the cornea are still major problems. Ulceration induced by persistent epithelial defects is often resistant to medical or surgical treatment<sup>2,3</sup>. When the basement membrane and stromal matrix are damaged, normal wound healing processes cannot proceed, and other factors, such as persistent inflammation, may also compromise wound healing<sup>2,3,4,5</sup>. Treatment of ulceration induced by persistent epithelial defects requires that a healthy basement membrane be provided and that inflammation be reduced to promote epithelialization<sup>2,3</sup>.

When persistent epithelial defect with ulceration develops, we often resort to such surgical therapies as tissue adhesive, conjunctival resection, lamellar keratoplasty, and penetrating keratoplasty<sup>4,5</sup>. The prognosis of penetrating keratoplasty in these inflamed eyes which frequently develop rejection<sup>6</sup>.

Amniotic membrane has long been used as a surgical material for ophthalmic surgery. De Roth first reported the use of amniotic membrane in conjunctival plastic surgery in 1940, but it has not been appeared again in the literature until recently<sup>2,3</sup>. The

current popularity of using amniotic membrane started when Kim and Tseng reintroduced the procedure in 1995. They reported that amniotic membrane transplantation offers a 40% chance of restoring corneal clarity and the epithelial phenotype in rabbits (n=13) in which the entire corneal and limbal surface had been experimentally destroyed, leading to a condition termed limbal stem cell deficiency<sup>4,5</sup>. Amniotic membrane has a number of indications, both as a graft to replace the damaged ocular surface stroma matrix and as a patch to decrease the inflammation<sup>2,3</sup>. In Indonesia the study of transplantation of human preserved human amniotic membrane on the ocular surface diseases is limited.

Based on this encouraging finding, the author want to report 2 cases of perforated bacterial corneal ulcer and one case persistent epithelial defect with ulceration, which were treated by transplantation of preserved human amniotic membrane, and to determine whether the preserved human amniotic membrane transplantation can also be used as an alternative method for treating perforated bacterial corneal ulcer. This experience will render a more appropriate treatment, a better cure rate and less complication.

## CASE REPORTS

### Case 1

A 38-year-old man developed an epithelial defect following *Pseudomonas aeruginosa* keratitis with hypopyon in the right eye one week before he developed a pseudomonas corneal ulcer, which was treated with topical antibiotics without success.

The epithelial defects progressed into stromal ulceration, which persisted for a week despite various treatments. His visual acuity was reduced to hand moving perception. The ulcer was in the central area. The operation was performed after informed consent.

Surgery was performed under local anesthesia by performing 3 ml retrobulbar injection and 2 ml parabolbar injection of total 5 ml solution of Lidocaine 2% in combination with Marcaine 0.75% (3:2). First, the bottom of the ulcer was debrided, and poorly attached epithelium at the edge of the ulcer was removed as bluntly as possible. After the corneal surface was treated and healthy corneal stroma was exposed, the first segment of amniotic membrane was transplanted as filling material in the stromal layer (amniotic membrane filling). The amniotic membrane was cut into small pieces and stuffed into the ulcer. The second amniotic membrane was transplanted as a basement membrane (amniotic membrane graft). Amniotic membrane was placed on the ulcer with epithelial side up and secured with 10-0 nylon sutures. The third amniotic membrane was transplanted as a cover (amniotic membrane patch) with 10-0 nylon sutures. The amniotic membrane patch was placed on the entire wound and corneal limbus with epithelial side up to protect the area of re-epithelialization. The technique of surgery in this case is overlay grafting.

Postoperatively, topical antibiotics, sytemic and topical anti fungus, and systemic non steroid anti inflammatory agent were administered. Topical antibiotics tarivid, thydin, gentamycin, cyprofloxacin were used 6 times a day. Systemic antifungus sporacid was used 3 times a day and topical anti-fungus diflucan was instilled every 10 minutes. Divoltar was orally given 3 times a day.

After amniotic membrane transplantation; however, the graft failure was detected 7 days later following corneal abcess, perforation and the prolaps of the iris. Topical antibiotics were administered continously. On the 16th day of the treatment, the prolaps of the iris was covered by epithelialization. Interestingly, despite absence of improvement of the visual acuity, the corneal ulcer completely healed 23 days after surgery.

### Case 2

A 72-year-old woman developed corneal ulcer with descemetocele in her left eye as a result of a Pseudomonas aeruginosa keratitis 2 months before visiting hospital. The visual acuity was reduced to light perception. The patient was treated by topical antibiotics, and the same procedure of amniotic membrane transplantation was performed on the 18th day of the treatment.

Amniotic membrane transplantation resulted in early evidence of epithelialization in 6 days, which

TABLE 1. -Patients profile

Case	Age (years)	Sex	Eye	Primary diagnosis
1.	38	Male	RE	Large corneal ulcer with hypopyon
2.	72	Female	LE	Large corneal ulcer with descematocele
3.	60	Female	LE	Persistent epithelial defect with central corneal ulcer

TABLE 2. -Treatment and clinical course

Case	Previous Treatment	PED (weeks)	Post op. follow up (weeks)	Healed (weeks)	Status of amniotic membrane
1.	Antibiotic Anti Fungus Patching	1	8	3	Dissolved in 3 weeks
2.	Antibiotic Anti Fungus Patching	1	8	6	Intact
3.	Antibiotic	3	8	4	Dissolved in 3 weeks

PED = Persistent epithelial defect

was complete in 6 weeks. The final visual acuity was still in light perception; however, the corneal ulcer did not result in perforation.

### Case 3

A 60-year-old woman developed central persistent epithelial defect and corneal ulcer in her left eye as result of *Staphylococcus epidermidis* infection suspected a month before visiting Dr Yap Eye Hospital. The visual acuity was reduced to 1/300. The patient was treated by ofloxacin 0.3% eye drops and 200 mg oral ofloxacin two times a day for 10 days. The clinical picture and the visual acuity was not improving. Preserved human amniotic membrane transplantation and tarsorrhaphy was performed. On the 10<sup>th</sup> day after transplantation, inflammation was reduced and the visual acuity was 3/60. The corneal ulcer was completely healed and the visual acuity was 6/30 on 28 days after surgery.

### DISCUSSION

Amnion is a collagen rich, thin, transparent, tough membrane lining the chorion leave and placenta that produces the amniotic fluid at the earliest period of fetus. Together the amnion and chorion compose the amniotic membrane<sup>4,5,7</sup>. The different between this study and the previously reported is the using of preserved membrane amniotic, not the fresh one. Some difficulties are found if fresh form is used in our hospital.

Previous studies have demonstrated that the amniotic membrane has unique properties. There are nine specific biological functions and benefits of amniotic membranes as wound dressings including the decrease of the bacterial count of the wound (antibacterial), reduction of fluid loss, promotion of healing, protection of growing epithelium, tight adherence to the wound surface, increase in mobility and diminished pain, help edition of readiness for grafting, preparation of skin defects for closure, decrease in physiological stress for the patient, stimulation of neovascularization, and fibrosis-suppressing effects<sup>2,5,8</sup>. These properties are considered suitable for the treatment of impaired epithelializa-

tion of the ocular surface. As mentioned before, Shuw-huey used fresh amniotic membrane for the treatment of persistent epithelial defects, and the results showed the efficacy of amniotic membrane patch<sup>4</sup>. Improvement of epithelialization may be attributed to inhibition of collagenase by amniotic membrane, and supplementation of the basement membrane and growth factors. In addition to the advantage of these properties, amniotic membrane was also used to supplement the collagen layer. A combination of collagen layer supplementation, basement membrane reconstruction, promotion of epithelialization and wound healing is required to treat severe ulceration.

Conservative management, such as continuous pressure patching with ointment, heals most cases of corneal epithelial defects. However, cases with persistent epithelial defects require more extensive procedures. Medical therapy with preservative-free lubricants as the most suitable agents is the treatment of first choice. Topical corticosteroid may be chosen to reduce inflammation but they should be used carefully.

There were two kinds of amniotic membrane grafting, such as: inlay and overlay grafting. Letko *et al* reported that was no difference between overlay and inlay techniques in terms of healing time and recurrence rate<sup>9</sup>. Overlay grafting technique was performed in all cases in ours study. The healing time was 18-36 days, the fastest of healing time was in case 1 which was the youngest. Based on previously reported the mean healing time was 25.5 days<sup>9</sup>.

Tarsorrhaphy is the first surgical technique in maintaining the moisture of the ocular surface to treat epithelial defects induced by a dry eye or inadequate blinking condition<sup>10,11</sup>. The efficacy of tarsorrhaphy is limited to the assistance of corneal wound healing. Prolonged, severe ocular surface disorders require more extensive treatment.

In the past, conjunctival transplantation or conjunctival cover was frequently used in an emergency, but it can cause neovascularization, corneal opacity, fibrosis and proliferation of abnormal epithelium<sup>12,13</sup>. Inflammation and invading vessel caused by conjunctival tissue may be a risk factor in further treatment, such as penetrating keratoplasty or other ocular surface reconstructions.

A donor cornea is the most suitable material for grafting to treat a damaged cornea. Penetrating keratoplasty and lamellar keratoplasty are commonly used to treat corneal ulceration<sup>12</sup>, but both require donor tissue. In emergencies, it is sometimes too late by the time a donor is found, especially in Indonesia, where the donor cornea supply is small. There is also the risk of rejection after surgery because of the donor cornea's immunogenicity.

Despite the absence of improvement of the visual acuity, the epithelization occurred in both patients and the perforated ulcers healed. As a wound dressing amniotic membrane has a number of benefits: in both patients the decreased inflammatory reaction diminished pain and increased ocular mobility, and may help in prediction of readiness for grafting.

## CONCLUSION

In summary, the author found that multilayered preserved human amniotic membrane transplantation may be effective for the treatment of deep corneal ulcer. This report showed the improvement of visual acuity in third case. Despite absence improvement of the visual acuity in the first and second case, the epithelization occurred in both patients and the perforated ulcers healed. The unique characteristics of amniotic membrane appear to offer a new surgical approach to ocular surface diseases.

## SUGGESTION

Consider on the failure, tarsorrhaphy should be considered. Further studies are needed to understand the mechanism involved in the benefits of amniotic membrane on the ocular surface reconstruction.

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