

# Multilayer vs. Monolayer Amniotic Membrane Transplantation for Deep Corneal Ulcer Treatment

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## ABSTRACT

*The purpose of the study was to evaluate the efficacy of multilayer amniotic transplantation (AMT) for reconstruction of corneal stroma and epithelium. Corneal ulcer (28) was a consequence of a previous infectious or neurotrophic keratitis. In the first group (17) ulcer was covered with monolayer AM, while in the other group (11) there were two or more layers of AM situated in the ulcer and the whole cornea was covered with AM sheet. Monolayer AMT was successful in 64% while the multilayer AMT success rate was 72%. AM gradually dissolved within 3–6 postoperative weeks. AM transplantation facilitates rapid healing of corneal epithelium, reduces inflammation and stimulates epithelial cell regrowth. In eyes with deep corneal ulcer multilayer technique proved to be better than monolayer procedure.*

## Introduction

Normal ocular surface is covered by corneal and conjunctival epithelium. Corneal epithelium is well known for its rapid self-renewal process. When normal healing of corneal epithelial defects is prevented, unique pathological state is manifested by poor epithelialization (persistent defects or recurrent erosions), chronic stromal inflammation (keratitis accompanied by scarring), corneal vascularization and conjunctival epithelial

ingrowth onto the corneal surface<sup>1,2</sup>. Numerous factors can cause these conditions, such as malfunction of lids and tear film, damage of corneal nerves, physical or chemical injuries, infections, systemic disorders<sup>2,3</sup>, as well as disorders that can cause scarring of the conjunctiva or persistent ocular irritation, primarily or secondary, involving the cornea<sup>4</sup>. If severe, disturbances of the ocular surface may lead to a significant visual impairment. It's been also shown that resident corneal cells when stimulated can produce spe-

cial proteins called chemokines that can lead to the recruitment of inflammatory cells into the tissue. It has been recognized that stromal keratocytes can transcribe and translate several chemokines, such as interleukin-8, RANTES, and MCP-1<sup>5,6</sup>. Moreover, collagenases produced by keratocytes and polymorphonuclears cause progressive corneal thinning with potential risk of corneal perforation and loss of the eye. Treatment of such patients is challenging, often unsatisfactory and requires combining approach.

Many treatments are used to treat these conditions. Some of them, such as padding with lubrication ointment and artificial tears are in general use. Conventional treatment of such diseases is aimed to eliminate the underlying disease mechanism as well as to control inflammation and to protect corneal surface. More invasive treatments such as superficial keratectomy, anterior stromal puncture or laser (Yag or excimer) therapy have also been used<sup>7–10</sup>. Recently, numerous papers have been reporting on the results of the mentioned techniques. In eyes with phenotypically abnormal corneal stroma and basal membrane (e.g. anterior stromal dystrophy, epithelial basement membrane dystrophy EBMD, abnormality of hemidesmosomes and loss of anchoring fibrils) and in eyes with loss or hypofunction of the stem cells (limbal deficiency) the conservative treatment will not be satisfactory<sup>8,9</sup>. Therefore, these conditions remain a therapeutic challenge often requiring surgical intervention<sup>11,12</sup>.

It has been shown that amniotic membrane transplantation on the corneal surface improves healing of epithelium defects by preventing inflammatory cell infiltration and by reducing apoptosis in keratocytes. AM is composed of one epithelial cell layer, basement membrane and avascular stroma. Epithelium produces basic fibroblast growth factor, he-

patocyte growth factor, and transforming growth factor  $\beta$ . Thick basement membrane serves as an ideal substrate to support growth of epithelial cells, since it facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells and promotes epithelial differentiation<sup>14,15</sup>. These reactions explain why amniotic membrane can be used to facilitate epithelialization in persistent epithelial defects. In addition, mentioned characteristics explain why AM can be used to expand remaining LSC (Limbal Stem Cells) since it can promote amplification of cells in cases of partial LSC (limbal stem cells) deficiency. Moreover, a component of corneal epithelial basement membrane collagen IV as well as metalloproteinase was identified in the avascular stroma.

Having in mind the healing properties of one layer AM in several diseases of ocular surface<sup>16</sup>, we wanted to determine whether its usefulness could be improved by the use of several layers for reconstruction of corneal stroma and epithelium in cases of deep stromal ulcers. Also, we wanted to compare this method with previously used monolayer technique. In this paper we compare multilayer and previously used monolayer technique for treatment of deep corneal ulcers.

## Patients and Methods

Twenty-eight patients with deep stromal ulceration unresponsive to all previous treatments were included in the study. Extensive stromal ulcerations persisted for at least several months. Amniotic membrane transplantation was performed in a period of 1999–2000. Preceding diagnosis, age and surgeries are listed in Table 1. Out of 28 patients 10 had previous infectious ulcer and 18 patients had neurotrophic keratitis. Patients were divided into two groups according to received treatment, monolayer

AMT (17) and multilayer AMT (11). All patients received treatment with artificial tears, ointment and eye patching prior to AMT. In two of them punctual plugs were placed due to the severe keratitis sicca. In the group with inflammatory keratitis antimicrobial therapy was administered according to swab finding. AMT was performed only in cases of negative control swab. In patients with exposure problems (2 patients in monolayer group and 1 patient in multilayer group) AMT was performed after plastic surgery. In cases of unsuccessful AMT surgery, second AMT was performed.

Human placenta was obtained in sterile conditions shortly after elective Caesarian section. It was screened against hepatitis B and C viruses, HIV and syphilis. The method of amniotic membrane preparation and preservation has been previously described in our work. Placenta was cleared from blood clots in standardized »washing solution« containing antibiotics. The isolated AM was flattened onto the nitroglycerine paper (size 3 × 3) with the epithelium/basement membrane side up and stored at –80 °C in the sterile bottles containing tissue culture (Inosol, Opsia, France) and glycerol at the ratio 1:1<sup>16</sup>.

All patients received parabolbar anesthesia (2 ml of 2% lidocaine) previous to surgical procedure. All debris, inflamed and fibrovascular tissue, together with the poorly adherent epithelium near the lesion, as well as the 1–2 mm zone around the edges of the ulcer were removed using Satin Crescent knife in order to create the firm base for attachment of the amniotic membrane and to allow re-epithelialization of the membrane from a neighboring healthy corneal epithelium. In the monolayer procedure AM was removed from the storage medium and transferred to the recipient eye. Edges were trimmed and one layer of AM was transferred to the eye with the base-

ment membrane side facing up. The whole cornea and limbus were covered and the membrane was sutured onto the conjunctiva using 10.0 nylon or Vycril sutures. Monolayer AM was not sutured to the cornea to prevent immunological reaction and vascularization of the cornea. In the multilayer procedure, first a large piece of AM was sutured to the upper 2/3 of the conjunctiva covering the whole cornea, then the stromal defect was filled with up to three layers of AM. If the layers were stable, sutures were not placed into the cornea. In cases of mobile multilayers, the most upper layer was secured to the cornea with interrupted 10–0 nylon sutures. Minimal number of sutures able to hold AM in place was used. After the cavity of the ulcer was filled and good adherence of the layers was secured, superficial layer of AM was sutured in the remaining 1/3 of the circumference to the conjunctiva with 10–0 nylon sutures. Postoperatively eyes were bandaged for two days and antibiotic and solutions were applied.

## Results

The decision for the AMT was made according to medical indications, after the failure of other conservative or surgical procedures.

In eyes where treatment with artificial tears and ointment was unsuccessful additional surgical procedures were performed: plastic surgery of the lids, in order to prevent lagophthalmos or trichiasis (2 patients from the monolayer group and 1 patient from the multilayer group) (Table 1), punctual plugs, in order to enable wetttable conjunctival and corneal surface (1 patient from the monolayer group). Before AM transplantation all patients with infectious ulcers were treated with antibiotic therapy till the causing agent was eradicated.

**TABLE 1**  
DIAGNOSIS AND PRECEDING SURGERY

Group	Patients (No.)	Diagnosis	Age (yrs)	Preceding Surgery
Monolayer	17	PIU (7)	20–80	PL (2)
		NK (10)		
Multilayer	11	NK (3)	26–75	PL (1)
		PIU (8)		

PIU – Postinfective ulceration, NK – Neurotrophic keratitis, PL – Plastic surgery

**TABLE 2**  
SURGICAL OUTCOME AFTER AMT

Group	Healed (No / %)	Healing time (weeks)	Healed after first surgery
Monolayer	11 / 64%	4–6	7 / 63%
Multilayer	8 / 72%	3–4	6 / 75%

**TABLE 3**  
VISUAL OUTCOME AFTER AMT

Group	Preoperative BCVA $\geq 0.3$ (No / %)	Postoperative BCVA $\geq 0.3$ (No / %)
Monolayer	4 / 23%	6 / 35%
Multilayer	2 / 18%	4 / 36%

BCVA – Best Corrected Visual Acuity

The same surgeon performed all surgical procedures. Out of 28 patients, 17 eyes received monolayer AMT and 11 patients multilayer AMT. The eyes were examined every day in the first 4–5 days and once a week thereafter. Obtained surgical results are listed in Table 2.

In the group of monolayer procedure AMT was successful in 10 (64%), out of 17 patients. In 6 eyes (60%) ulcer has healed after first transplantation, while in 4 patients (40%) second transplantation was necessary. In multilayer procedure group, transplantation was successful in 8 (72%) out of 11 patients after the first surgery, while in 2 cases it had to be repeated. These results implicate better effectiveness of multilayer technique as compared to the monolayer technique.

In spite of the improved overall eye condition the majority of patients in both groups retained the same BCVA. In the monolayer group BCVA of # 0.3 was recorded in 4 eyes (23%) before transplantation and in 6 eyes (35%) after transplantation. In the multilayer group the same BCVA was recorded in 2 eyes (18%) before transplantation and in 4 eyes (36%) after transplantation. Visual improvement after surgery did not differ between the two groups. The results are listed in Table 3.

## Discussion

Amniotic membrane in the eye was primarily used as an alternative allograft to replace the lack of conjunctiva in cases

of surgically created large conjunctival defects (dysplasia, scars, tumors, and symblepharon)<sup>25</sup>. Impression cytology has proved that few weeks after AMT epithelial phenotype covering AM is conjunctival, with significantly higher density of epithelial and goblet cells as compared to conjunctival tissue transplantation<sup>18</sup>. These results were attributed to the unique, but at that time still unknown, composition of AM<sup>17</sup>. Recently, several components and possible mechanisms of action of AM have been recognized. Basement side of the membrane has proved to be an ideal substrate for epithelial progenitor cells. It prolongs their life span and maintains their clonogenicity. Another characteristic of amniotic membrane is that it promotes non-goblet differentiation of the conjunctival epithelium<sup>18</sup>. Eyes with persistent ulceration are exposed to pathological conditions for longer period of time and stem cells are usually partly or totally destroyed<sup>19–21</sup>. Therefore, the ability of AM to enhance growth of progenitor cells might overcome the lack of stem cells.

Some other ocular disturbances like peripheral ulcerative and inflammatory keratitis, limbitis or neurotrophic and ischemic keratitis do not directly destroy limbal stem cells, but instead damage the limbal stroma so that it cannot support growth of the stem cells<sup>21</sup>. For partial limbal deficiency with superficial involvement, amniotic membrane transplantation can support growth of the cells by reducing the vascularization and preparing the stroma for epithelial regrowth. AMT for epithelium regrowths has been advocated by some authors as a sufficient and even superior to allograft limbal trans-

plantation (ALT) because it does not require administration of cyclosporin. In cases of total limbal deficiency, additional ALT is needed. It has been shown that AMT enhances successful engraftment of ALT by preparing the perilimbal stroma and reducing inflammation and vascularization<sup>22–24</sup>.

Although most surgeons suture monolayer AM to the cornea, we have used monolayer without suturing to the cornea, but instead covering the whole cornea. In this manner the effect of the basement membrane as a replacement for the damaged Bowman's membrane was minimized, but the distinctive properties of the AM stroma were preserved. It was recognized in several studies that stromal matrix contains specific compounds that can suppress TGF-beta (Transforming Growth Factor) signaling, proliferation and myofibroblast differentiation of normal corneal and limbal fibroblasts<sup>25</sup>. This explains why AMT reduces scarring during conjunctival surface surgeries. Such an action is more potent when fibroblasts are in contact with the stromal matrix, and weaker when fibroblasts are distant from the membrane. This effect implies that some diffusible factors might also be secreted by stromal matrix, and some of them were identified as growth factors. Moreover, stromal matrix containing various forms of protease inhibitors can decrease inflammatory cell population<sup>5,25</sup>. The unique composition of AM seams in fact to work as a natural »healing patch« for corneal ulcers<sup>26,27</sup>. The use of multilayer AM can reinforce the healing effect of AM by amplifying the concentration of useful factors secreted from the stroma.

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## USPOREDBA USPJEŠNOSTI TRANSPLANTACIJE VIŠESLOJNE I JEDNOSLOJNE AMNIJSKE MEMBRANE U LIJEČENJU DUBOKIH ULKUSA

### SAŽETAK

Cilj rada bio je ustanoviti je li transplantacije višeslojne amnijske membrane prilikom liječenja dubokih ulkusa rožnice učinkovitija od jednostruke transplantacije. Rožnični ulkus (28) nastao je kao posljedica recidivirajućih infekcija ili neurotrofičkih keratitisa. U prvoj grupi ispitanika (17) rožnica je bila pokrivena jednostrukom amnijskom membranom, dok su u drugoj skupini (11) presađena dva ili više slojeva AM u sam defekt, a cijela je rožnica bila dodatno pokrivena jednim slojem amnija. Membrana se postupno rastopila unutar 3–6 postoperativnih tjedana. Jednostruka transplantacija bila je uspješna u 64% slučajeva, dok je nakon presađivanja više slojeva do izlječenja došlo u 72% ispitanika. Istraživanje je pokazalo da je u liječenju dubokih ulkusa rožnice uspješnija metoda presađivanja višeslojne amnijske membrane.