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Amniotic Membrane Transplantation in the Treatment of Persistent Epithelial Defect on the Corneal Graft

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A B S T R A C T

It has been shown that amniotic membrane transplantation (AMT) improves healing of the epithelium defects as it serves as a basement membrane for endothelial cells growth, prevents inflammatory cell infiltration and reduces apoptosis in keratocytes. Having in mind the healing properties of AM we investigated the efficacy of AMT in persistent epithelial defect (PED) on the corneal graft. 80 corneal grafts were prospectively followed up for presence of PED 10 months after surgery. PED was detected in 12 cases (15%) having surgery for: rejected graft (n=4), keratoconus (n=3), keratoconus following PK on a second eye (n=3), corneal perforation (n=1) and Stevens-Johnson keratopathy (n=1). Epithelial defect (ED) developed 14±7 days after surgery in 10 cases and 1,5 month in other two. All patients were primarily conservatively treated with subconjuctival steroids and artificial tears for 10 days and systemic steroid therapy if needed after, until the period of 2 weeks. 4 patients were healed. Since ED was unresponsive to all previous treatments for more then 2 weeks, one layer of AM was placed on the corneal lesion in 5 patients, and in 3 cases of deep PED several layers of AM were placed. Healing of the defect was obtained in 7/8 (87.5%) eyes. In 1 patient second AM transplantation was necessary. Mean epithelization time was 2 weeks (range 1–3 weeks) in monolayer and 3 weeks (range 2–4 weeks) for multilayer cases. 5 out of 8 patients retained the same best corrected visual acuity (BCVA) while 3/8 patients improved their vision more than 2 lines. Preoperative corneal thickness of 255±40 mm increased to 455±90 mm. AM transplantation facilitates healing of corneal epithelium. PED on the corneal graft unresponsive to conventional treatment can be effectively cured when covered with one or more amniotic membrane layers.

***Key words**: amniotic membrane transplantation, corneal graft, persistent epithelial defect*

Introduction

The human amniotic membrane (AM) is the innermost layer of the placenta and consists of a single epithelial layer, a thick basement membrane and an avascular stroma. Due to the number of its properties, AM is increasingly used in the treatment of severe ocular surface diseases[1]. The amniotic basement membrane facilitates migration and growth of epithelial cells, therefore promoting epithelialization. The avascular stroma of the AM reduces fibrovascular ingrowth and abnormal neovascularization. Amniotic epithelium produces anti-inflammatory and growth factors beneficial to the treatment of inflammatory corneal diseases[2–4]. Human amniotic membrane epithelial and mesenchymal cells express various antiangiogenic and anti-inflammatory proteins such as interleukin(IL)-1 receptor antagonist and IL-10, basic fibroblast growth factor, hepatocyte growth factor and transforming factor β, facitilitate the migration of epithelial cells, the reinforcement of basal cellular adhesion and the encouragement of epithelial differentiation. In the successful reconstruction, the epithelial cells are covering the amniotic membrane and conjuntival and corneal surfaces are stabile, wettable noninflamed and free of fluorescein staining, although the exact mechanism achieving these results remains unclear[2]. It has been shown that amniotic membrane attracts and traps inflammatory cells infiltrating the ocular surface, which may explain some of its anti-inflammatory properties[5]. Corneal epithelium is well known for its rapid

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self-renewal process. When normal healing of corneal defects is prevented, unique pathological state is manifested by poor epithelisation (persistent defects or reccurent erosions), chronic stromal infiltration (keratitis accompanied by scarring), corneal vascularization and conjunctival epithelial ingrowth onto the corneal surface[6–8]. 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[6,8,9], as well as disorders that can cause scarring of the conjunctiva or persistent ocular irritation, primarily or secondary, involving the cornea[6,10]. Resident corneal cells when stimulated can produce special proteins called chemokines that can lead to the recruitment of inflammatory cells into the tissue and stromal keratocytes can transcribe and translate several chemokines, such as interleukin 8, RANTES and MCP1[6,11,12]. Moreover, collagenases produced by keratocytes and polymorphonuclears cause progressive corneal thinning with potential risk of corneal perforation and loss of the eye. In the transplant patient, this process is yet more complicated, since in the early postoperative period, there are additional insults of the relative denervation of the cornea, poor lubrication, the instillation of frequent and often toxic topical medications, and sometimes abnormal lid-cornea anatomical relationship[13]. Punctate erosions and vortex keratopathy, along with other types of epithelial abnormalities, are common after keratoplasty, especially in the early postoperative period[14,15]. If not appropriately managed, these ubiquitous problems can escalate into conditions that may threaten the health of the transplant. The critical period for stabilization of most surface problems is in the first 3 months[13]. Therefore, treatment of patients with corneal graft ulceration is challenging and requires combining approach. AMT offers the following advantages over corneal tissue use in the treatment of persistent epithelial defects: avoidance of potential allograft rejection and postoperative astigmatism of tectonic corneal grafts, ease and convenience of use, feasibility in the event of corneal tissue shortage and preservation of a better aesthetic appearance. The unique composition of AM seams in fact to work as a natural »healing patch« for corneal ulcers[6,16,17]. In this study we wanted to determine the efficacy of amniotic membrane transplantation (AMT) for the treatment of persistent corneal epithelial defects (PEDs) in eyes after penetrating keratoplasty (PK).

Materials and Methods

80 corneal grafts were prospectively followed up for presence of PED 10 months after surgery. PED was detected in 12 cases (15%) having surgery for: rejected graft (n=4), keratoconus (n=3), keratoconus following PK on a second eye (n=3), corneal perforation (n=1) and Stevens-Johnson keratopathy (n=1). Epithelial defect (ED) developed 14±7 days after surgery in 10 cases and 1.5 month in other two. ED conservative treatment with subconjuctival steroid, artificial tears and systemic steroid therapy if needed was administered in all patients. The decision for the AMT was made according to the medical indications after the ED was unresponsive to conservative treatment for more than 2 weeks in 8 patients. Preoperatively corneal epithelial lesions were stained with fluorescein and photodocumented by digital slit lamp (CSO Digital vision). Corneal thickness was measured with non-contact optical coherence tomography customized for the anterior segment (Zeiss Visante™ OCT) and the best corrected visual acuity (BCVA) was determined. Amniotic membrane (one or more layers) was placed on the corneal lesion, trimmed and sutured with resorptive continuous suture onto the surrounding conjuctiva. In 3 cases of deep PED several layers of AM were placed and sutured to its uppermost layer to prevent placement of sutures into cornea. Postoperatively eyes were bandaged with contact lens and topical steroid/antibiotic/artificial tears were applied. Patients were checked on the first postoperative day and then on the weekly basis for the epithelial size defect, corneal thickness and finally BCVA after AM melting.

Amniotic membrane preparation

Amniotic membranes (AM) were obtained from the certified eye bank (Vseobecnà fakultní nemocnice, Prague (OTB) where they were prepared under sterile conditions from a human placenta shortly after elective caesarean delivery. Informed consent was obtained from each donor, and screening was made to exclude any risk of transmissible infections such as human immunodeficiency virus, hepatitis virus types B and C, and syphilis. The AM was cut in 3 cm × 4 cm pieces, flattened onto individually sterilized nitrocellulose paper without folds or tears with the epithelial surface up. Each AM was delivered in a sterile vial containing tissue culture and glycerol at a ratio of 1:1in a frozen stage.Before use in the operating theatre, AM was thawed by leaving the vial at room temperature, and then the membrane is transferred to the ocular surface.

Surgical procedure

Previous to surgical procedure all patients received subconjunctival or parabulbar anesthesia (2 mL of 2% lidocain). In the beginning of the surgical procedure all poorly adherent epithelium near to the lesion was removed using Satin Crescent Knife in order to create the firm base for attachment of the AM and to allow reepithelisation of the membrane from 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 recipient eye with the basement membrane side facing up. The whole cornea and limbus were covered without folds and tears and sutured by running 10.0 resorptive sutures (Biosorb). 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 stabile, additional sutures were

not placed. In the cases of mobile multilayers, membranes lying onto the PED were secured to the most upper layer of AM with 10.0 resorptive sutures. Minimal number of sutures able to hold AM in place was used. After the cavity of the deep PED 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 resorptive sutures. A soft contact lens is placed over the membrane at the end of the surgery.

All surgical procedures were performed by the same surgeon.

Results

Conservative treatment of the epithelial defect with subconjuctival steroid/artificial tears and systemic steroid therapy was obtained in 4 patients (3 keratoconus and 1 keratoconus following PK on second eye patients). 7 out of 8 patients, unresponsive to previous conservative therapy, obtained healing of the defect with AMT. In one patient having previously rejected corneal graft, second AM transplantation was necessary. In 3 cases of deep PED (2 keratoconus patients following PK on the 2nd eye and 1 patient with rejected graft) several layers of AM were placed. Mean epithelization time was 2 weeks (range 1–3 weeks) in monolayer (Figure 1) and 3 weeks (range 2–4 weeks) for multilayer cases. Epithelial defects situated peripheraly on the cornea healed faster. Five out of eight patients retained the same BCVA while 3/8 patients improved their vision more than 2 lines (2 keratoconus patients and 1 with previously rejected corneal graft). Mean preoperative corneal thickness of 255±40 μm increased to 455±90 μm in cases with deep PED (Figure 2).

Discussion

Amniotic membrane transplantation has gained widespread attention as an effective method of reconstruction of the ocular surface, having a unique combination of properties, including the facilitation of migration of epithelial cells, the reinforcement of basal cellular adhesion and the encouragement of epithelial differentiation. Its ability to modulate stromal scarring and its anti-inflammatory activity has led to its use in the treatment of ocular surface pathology. Amniotic membrane transplantation has been used for reconstruction of the corneal surface in the setting of persistent epithelial defects, partial limbal stem cell deficiency, bullous keratopathy and corneoscleral ulcers. It has also been used in conjunction with limbal stem cell transplantation for to-

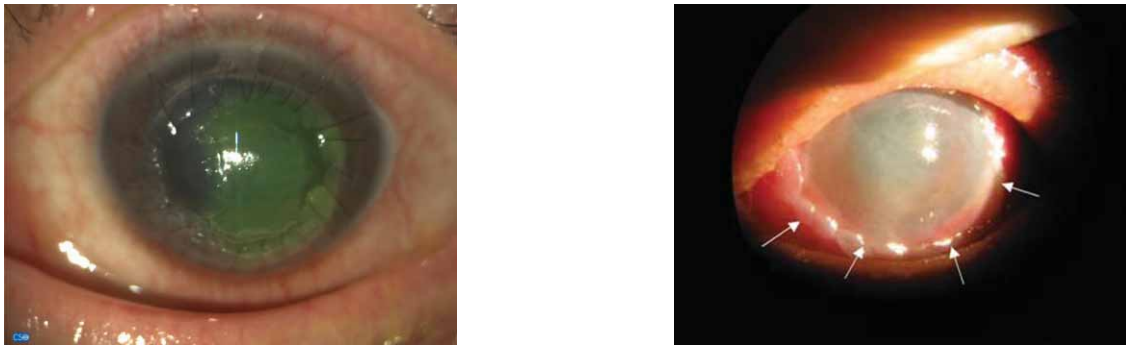

Fig. 1. A. Epithelial defect on the corneal graft before AMT, B. Monolayer AMT placed on the epithelial defect of the corneal graft, the border of AM indicated by arrows. AM-amniotic membrane, AMT-amniotic membrane transplantation. (jpg files)

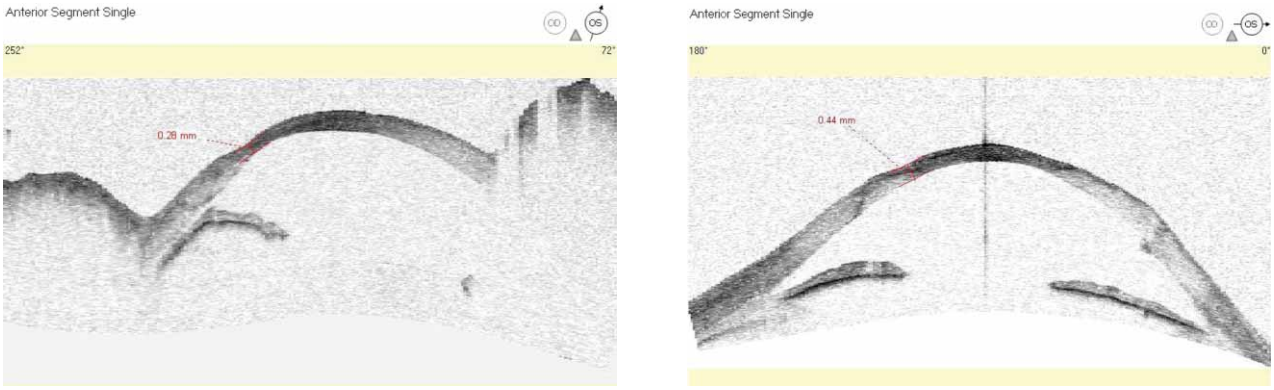

Fig. 2. A. Visante OCT preoperative corneal thickness with deep PED, B. Visante OCT postoperative corneal thickness with deep PED. Visante OCT-optical coherence tomography customized for the anterior segment, PED-persistent epithelial defect. (jpg files)

tal limbal stem cell deficiency[18]. In persistent epithelial corneal defects, AM serves to provide a basement membrane substrate for the migration and adhesion of epithelial cells when used as an inlay graft. When used as an overlay patch it facilitates epithelialization by providing a barrier against inflammatory cells and mediators. The AM, being continuously moistened by tears, provides adequate hydration to the regenerating epithelium and protects it from the abrasive effect of an abnormal palpebral conjunctiva[19]. The amniotic membrane stromal matrix has a direct anti-scarring effect as evidenced by its suppression of TGF-β signalling and myofibroblast differentiation, helps re-establish a microenvironmental niche that is conducive for the growth of epithelial progenitor cells.Finally, the amniotic membrane may promote nerve regeneration by maintaining nerve growth factor (NGF) signaling[20]. Amniotic membrane transplantation may be considered an alternative method for treating PEDs that are refractory to conventional treatment[21]. In our study we wanted to determine AM healing effects on the epithelial defects of corneal grafts. PED developed in 15% of all transplants in average of 10 days after surgery with exception of the two later cases where it developed after 1,5 month. Four patients had previously rejected corneal grafts, and three had keratoconus with a corneal graft on the other eye. The remaining others had epithelial defects on the keratoconus cornea, one had corneal perforation and one Stevens-Johnson syndrome. In 4 patients conservative treatment of ED with subconjuctival steroid/artificial tears, therapeutic contact lens and systemic steroid therapy was effective. In the other 8 patients conservative therapy failed and AMT (5 monolayer and 3 multilayer) was performed after two weeks. ED healed in average in 2 weeks (range 1–3 weeks) in monolayer and in 3 weeks (range 2–4 weeks) for multilayer cases. In patient who had previously rejected corneal graft we had to perform second AMT transplantation. Patients with keratoconus and peripheral lesions healed faster than the others. Patients who had previously transplanted and rejected graft and also the patients who had previously transplanted the other keratoconus eye had deep PED and several layers of AM were sutured. Their visual acuity improved for two lines after the surgery. In these cases the use of multilayer AM can indeed reinforce the healing effect of AM probably by amplifying the concetration of useful factors secreted from the stroma. In one patient with Stevens-Johnson syndrome (SJS) AMT very successfully healed ED and improved BCVA. AMT is established method of therapy for SJS and can avert the catastrophic ophthalmic sequelae of this rare but devastating disease[22–24]. Corneal thickness of patients with deep PED was thicker for 200 μm in average as compared to before AMT. Persistent epithelial defects can compromise ocular surfaces and deplete the stem-cell population that repairs the damaged corneal epithelium, leading to pain, scarring, vascularization, and loss of sight. Their treatment includes: elimination of underlying problems, control of inflammation, prevention of additional loss of tissue, protection of ocular surfaces with a bandage contact lens and possible surgery or corneal transplant. When medical therapies fail, the type of surgery (e.g., ocular surface reconstruction) indicated is dependent upon the »extent of involvement of the cornea (e.g., epithelium, basement membrane or stroma), extent of limbal ischemia, conjunctival necrosis and intact tear supply«[25,26]. Amniotic membrane (AM), the innermost layer of the fetal membrane, exhibits properties that are helpful in wound healing, particularly of ocular injuries and has been proposed for various clinical indications for ocular reconstruction. We conclude that persistent epithelial defects on the corneal graft unresponsive to conventional treatment can be effectively cured when covered with one or more amniotic membrane layers.

Abbreviations: AMT – Amniotic membrane transplantation, AM – Amniotic membrane, PED – persistent epithelial defect, ED – Epithelial defect, BCVA – best corrected visual acuity

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TRANSPLANTACIJA AMNIJSKE MEMBRANE U LIJEČENJU PERZISTENTNIH EPITELNIH EROZIJA ROŽNIČNOG TRANSPLANTATA

S A Ž E T A K

Istraživanje su pokazala da transplantacija amnijske membrane (TAM) poboljšava cijeljenje epitelnih defekata tako da služi kao bazalna membrana za rast endotelnih stanica, spečava infiltraciju upalnih stanica i smanjuje apoptozu u keratocitima. Imajući na umu cijeljeće učinke amnijske membrane (AM) istraživali smo uspješnost TAM u perzistentnim ne-cijeljećim defektima (PND) rožničnih transplantata. Osamdeset pacijenata prospektivno je praćeno u razdoblju od 10 mjeseci nakon operacije. PND je imalo 12 pacijenata (15%) koji su imali transplantaciju rožnice zbog: prethodno odbačenog transplantata (n=4), keratokonusa (n=3), transplantacije na drugom oku zbog keratokonusa (n=3), puknuća rožnice (n=1) i Stevens-Johnsonova sindroma (n=1). Epitelni defekt (ED) se pojavio 14±7 dana nakon operacije u 10 pacijenata a 1.5 mjeseci nakon operacije u preostala dva. Svi su pacijenti primarno liječeni konzervativno kortikosteroidnim subkonjunktivalnim injekcijama i umjetnim suzama kroz 10 dana i po potrebi sistemskom steroidnom terapijom u trajanju do 2 tjedna. U četiri pacijenta ED je zacijelio na lokalnu terapiju. U 5 pacijenata kod kojih ED nije cijelio na odgovarajuću terapiju više od 2 tjedna jedan sloj amnijske membrane je transplantiran na defekt rožnice, a u 3 pacijenta koja su imala duboki ED višeslojna amnijska membrana je stavljena. Cijeljene defekta je nastalo u 7/8 (87,5%) pacijenata. U jednog pacijenta bila je potrebna druga TAM. Prosječno vrijeme epitelizacije bilo je 2 tjedna (1–3 tjedna) kod tranplantacije jednog sloja AM dok je u višeslojne iznosilo 3 tjedna (2–4 tjedna). U pet od osam pacijenata vidna oštrina ostala je jednaka kao prije transplantacije dok se u 3/8 pacijenata vidna oštrina popravila za više od dva reda na Snelenovim tablicama. Preoperativna debljina rožnice od 255±40 μm porasla je na 455±90 μm. Transplantacija AM pomaže cijeljenju epitela rožnice. Duboki epitelni defekt transplantata koji ne cijeli na standardnu terapiju može se uspješno liječiti tranplantacijom jednog ili više slojeva amnijske membrane.