Literature Summary on Uses of AmnioGraft in Ocular Surface Reconstruction

(By Dr. Scheffer CG Tseng)

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I. Introduction

Amniotic membrane, the innermost layer of fetal (or placental) membrane, consists of a simple epithelium, a thick basement membrane and an avascular stroma. Its function is to protect the fetus from unwanted maternal insults during development. It has been recognized that the incision at the skin of the fetus during fetal surgery does not bear any scarring after birth. The phenomenon of "scarless fetal wound healing" remains a mystery[1, 2] . It is tempting to speculate that the amniotic membrane carries the same feature as the fetal tissue in exerting scarless fetal wound healing.

A number of clinical studies have shown that amniotic membrane covered corneal or conjunctival surfaces show rapid epithelialization (i.e., covering of the epithelial cells onto the denuded amniotic membrane) and reduced inflammation, scarring, and unwanted blood vessels. In other words, amniotic membrane facilitates healing and regeneration of cells and tissues with minimal inflammation and scarring [for reviews see [3-6] . [7-11] , [12-16] and a recent editorial [17]].

II. Action Mechanisms

There are a number of action mechanisms that have been put forth to explain the effectiveness of amniotic membrane used in ocular surface reconstruction. These actions have recently been reviewed [18] . They are:

- The amniotic membrane's basement membrane contains type IV collagen, laminin 1, laminin 5, and collagen VII. Among the six known chains of the collagen IV, the α2 chain is found in human amniotic membrane, a pattern similar to that of the conjunctiva[19] , the α3 chain is found in rat amniotic basement membrane[20] , and the α5 chain of the type IV collagen is found in human amniotic basement membrane [19, 21] , like that of the corneal epithelial basement membrane. Recently, our laboratory discovered that besides α2 and α5 chains, the human amniotic membrane also express α1 chain (Li et al, manuscript in preparation, 2005). Based on these data, we may conclude that the human amniotic basement membrane contains $[(\alpha1)_2\alpha1]$ and $[(\alpha5)_2\alpha6]$ triple chains. It has been shown that laminin 5 is important in facilitating corneal epithelial cell adhesion.[22] . Collagen VII found in human amniotic membrane [19] forms anchoring fibrils that help stabilize epithelial adhesion. Therefore, the basement membrane side of amniotic tissue may be an ideal substrate for supporting the growth of epithelial basal progenitor cells. Epithelial cells, including stem cells, anchor to the basement membrane. Furthermore, the basement membrane facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells, and promotes epithelial differentiation, and prevent epithelial apoptosis, i.e., programmed cell death[23, 24] .

- The amniotic membrane's stroma contains unique extracellular matrix components such as fibronectin [19] , type I [25] and type III collagens [26] , type IV collagen [27] , and such glycosaminoglycans/proteoglycans as hyaluronic acid, decorin, and biglycan, and non-glycosylated lumincan, which can facilitate epithelial wound healing [28] . The functions of these matrix proteins remain to be elucidated.

- The amniotic membrane contains several mitogenic growth factors [29] , several anti-angiogenic and anti-inflammatory proteins [30] [31] , and natural inhibitors to various s proteases. [32-34] Among mitogenic growth factors, it is particularly intriguing to note that amniotic membrane contains several neurotrophins[35] and nerve growth factor (NGF)[36] , while this tissue is not innervated. The presence of IL-10 in amniotic membrane [30] [37] [38] may help explain its anti-inflammatory action. The anti-angiogenic action of amniotic membrane may in part be explained by the expression of pigment epithelium derived growth factor (PEDF) [39] and endostatin [31] , which is found in the basement membrane and after degradation by MMP, respectively.

- Although the exact molecular candidate(s) in amniotic membrane remains to be elucidated, several experimental studies have demonstrated that amniotic membrane indeed exerts potent anti-inflammatory actions. Expression of IL-1α and IL-1β is markedly suppressed when human limbal epithelial cells are cultured on the amniotic membrane stromal matrix, even where challenged by lipopolysaccharide.[40] . When rabbit's corneas receive excimer laser ablation to remove the basement membrane and the superficial stroma in a procedure called superficial keratectomy, known to elicit the least amount of host inflammation. If such a wound is covered by one layer of amniotic membrane, it has been reported that acute inflammation is reduced as evidenced by the rapid apoptosis of polymorphonuclear neutrophils[41] .[41, 42] This finding was supported in human patients with acute burns where CD20+ cells are trapped by amniotic membrane and exhibited apoptosis.[43] When rabbit's corneas are injured by alkali, amniotic membrane transplantation reduces acute and severe inflammation as evidenced by less amount of infiltration of polymorphonuclear neutrophils.[44] When rat's corneas are inoculated with HSV-1 (herpes simplex virus type 1) to elicit a severe form of necrotizing keratitis with intense acute and chronic inflammation, it has also been reported that such an inflammation is reduced by covering with one layer of preserved human amniotic membrane for two days[45] The infiltration of polymorphonuclear neutrophils, lymphocytes, and marcrophages is all reduced [45] with rapid apoptosis (manuscript submitted, 2005). In culture, recently, it has been shown that human amniotic membrane stromal matrix facilitates apoptosis of murine macrophages, especially when they are activated by IFN-γ [46] . Collectively, the above findings provide evidence to support the anti-inflammatory effect of amniotic membrane transplantation.

- Although the aforementioned amniotic membrane's anti-inflammatory actions

may indirectly contribute to its anti-scarring actions, several lines of experimental evidence also support the notion that amniotic membrane may exert a direct anti-scarring effect on fibroblasts. Although the exact molecular candidate(s) in the amniotic membrane remains to be determined, it has been well demonstrated that the amniotic membrane stroma suppresses TGF-β signaling and myofibroblast differentiation for cultured human corneal fibroblasts and limbal fibroblasts[47] and cultured human conjunctival fibroblasts and pterygium body fibroblasts.[48] Expression of TGF-β1 and deposition of collagen and fibronectin are reduced by amniotic membrane transplanted on excimer laser ablated corneal surface.[49, 50] Human amniotic membrane transplantation into the rabbit corneal stromal pocket reduces the myofibroblast differentiation elicited by invading epithelial cells and in a tissue culture model of collagen gel contraction.[51] These action help explain why corneal scarring (also termed haze) is reduced in excimer laser-induced keratectomy in rabbits by amniotic membrane transplantation[41] .[41, 52-54] Collectively, these experimental data support the notion that amniotic membrane transplantation reduces scar formation, i.e., possessing an anti-scarring effect.

- Recently, it has been reported that amniotic stromal matrix is capable of maintaining the characteristic dendritic morphology and keratocan expression of human [55] [56] , murine [57] , and monkey (manuscript submitted, 2005) keratocytes in culture while continuing expanding these cells even in the presence of serum. This unique action of amniotic membrane stroma matrix is closely linked to its effect of downregulating TGF-β signaling including downregulation of TGF-β promoter activities[56, 57] .

- The combination of the aforementioned effects also explains why amniotic membrane has been used as a ideal substrate to cultivate epithelial progenitor cells of the conjunctiva [58-60] }[59, 61] , the cornea[62, 63] , the limbus [61, 64-70] , the oral mucosa [71, 72] [63] , and the corneal endothelium[73] *in vitro.* The resultant cultivated cells and amniotic membrane have been transplanted in normal rabbits, [74] [71] as well as in limbal stem cell deficiency rabbit [71, 75] and rat models, for a short-term study [76] . It also has been used in limbal deficient rabbits for a long-term study [77-79] . Currently this composite tissue is being transplanted to restore vision as well as the structure and the function of damaged ocular surfaces in humans[80]
[61]
[81, 82] [72] . At the present time, there are several experimental protocols used to expand limbal epithelial progenitor cells using amniotic membrane. They vary in the use of limbal epithelial explants or dispase-isolated epithelial cell suspension, the use of intact or denuded amniotic membrane, and the use of 3T3 fibroblast feeder layers. It remains unclear which protocol is mostg appropriate and effective in accomplishing this objective. These clinical effects are based on the premises that amniotic membrane facilitates epithelialization, prolongs epithelial stem cell survival, and reduces inflammation and scarring.

III. Clinical Uses

Clinical uses of amniotic membrane transplantation can be grossly categorized into two major modes, i.e., as a temporary graft or as a permanent graft. For the latter, it can be further categorized into two major modes, i.e., to be used alone or in conjunction with other surgical procedures.

When amniotic membrane is used as a *temporary graft*, the membrane is sutured as a bandage (lens), dressing, or patch to cover both healthy host tissue and the site of interest at the same time so that host epithelial healing heals underneath. Upon completion of epithelialization, the membrane is invariably dissolved or removed in a period varying from 2 to 6 weeks. When amniotic membrane is used as a *permanent graft,* the membrane is sutured to fill in the tissue defect of the cornea or the conjunctiva so that host cells will grow over or into the membrane and the membrane will be integrated into the host tissue.

1. As a Temporary Graft (Patch, Dressing, or Bandage)

As stated above and summarized in Fig. 1 and in a recent review[10, 11] , AM can be used as a *temporary* graft (patch, dressing or bandage) to reduce inflammation and scarring of the ocular surface and hence reduce the pain and discomfort experienced by the patient inflicted with various diseases and insults. They include acute chemical and thermal burns of the ocular surface [44, 83-90] which remain as one of the most devastating and challenging ophthalmic emergencies. Chemical burns, especially alkali, result in severe inflammation, which if left relentless and chronic, invariably results in granulation and scarring. Scars on the corneal surface threaten the vision, scars in the conjunctiva cause motility restriction, and scars in the lids lead to exposure, mechanical micro-trauma (by misdirected lashes and keratinization), and dryness. Conventional therapies for chemical burns have shown a limited success. AM transplantation as a temporary graft has been shown to reduce inflammation and scarring, hence facilitating wound healing and restoring comfort and vision. Thus, Pan et al [89] reported the therapeutic effect of amniotic membrane transplantation in 28 eyes with ocular burns; chemical (n=20) and thermal (n=8). In all eyes, the ocular inflammation was controlled after 3-7 days of surgery. There was corneal transparency in 16/28 eyes. All eyeballs were saved and had stable ocular surface.

AM can also be used to suppress inflammation, promote healing, and prevent scarring in patients suffering from acute Stevens Johnson syndrome (SJS) with or without toxic epidermal necrolysis (TEN) at the acute stage[91, 92] (Kobayshi et al, 2005, in press). The conventional managements at Intensive Care and Burn Units are directed to life-threatening problems, and thus frequently are inadequate to address the ocular inflammation and ulceration. As a result, patients suffering are frequently left with a blinding disease because of scarring-induced late complications.

AM as a temporary graft can also be used to treat chronic recalcitrant inflammation with or without persistent epithelial defect caused by various diseases [93] including neurotrophic keratitis caused by herpes zoster virus (HZO) or herpes simplex virus (HSV)[17, 45 94] , vernal keratoconjunctivitis[8-10, 95, 96] . Occasionally, both a patch and

a graft are used together, and in that case the patch is used as a protective shield to ensure epithelization of the AM used as a graft [10, 11, 97] . Recently, AM has been advocated to promote healing and reduce inflammation in high-risk eyes receiving penetrating or lamellar keratoplasties.[94]

Furthermore, in experimental rabbit models, AM as a temporary graft is effective in reducing inflammation and corneal haze induced by excimer laser ablation [41, 41, 42, 42, 52, 98, 99] , and to improve implantation of keratoprosthesis, i.e., artificial cornea[100] (Fig. 3). However, as there are few reports, the clinical efficacy in human patients has not been thoroughly demonstrated. One of them is by Lee et al [54] , who reported 2 patients who developed subepithelial opacities and myopic regression after photorefractive keratectomy (PRK). Both cases were treated successfully with PRK and amniotic membrane application. Dua et al [10] used amniotic membrane after PRK in one patient with marked haze; repeat PRK was carried out with amniotic membrane application. The patient had minimal post-operative pain and epithelialization occurred within 5 days. The haze was reduced 50% but not enough to improve his quality of vision.

Fig. 1

Demonstrated in Human Patients:

- Acute Chemical or Thermal Burns
- Acute Stevens Johns Syndrome or Toxic Epidermal Necrolysis
- Chronic HZO or HSV Keratitis
- Persistent Epithelial Defects
- Shield Ulcers due to Vernal Keratitis
- High-risk Corneal Grafts

Demonstrated in Experimental Animals:

- Excimer Laser Ablation (PRK/PTK)
- Implantation of Keratoprosthesis

Since April 2005, Bio-Tissue, Inc. has made available a sutureless AmnioGraft, termed “ProKera”, through which AmnioGraft can be delivered to the ocular surface through a symblepharon ring (conformer). Such an FDA approved medical device is most ideal for accomplishing the above objectives without sutures.

2. As a Permanent Graft

Conventionally, when amniotic membrane is used as a permanent graft, it is secured onto the ocular surface by interrupted or running sutures. New advances have been made to reduce the surgical time and to improve the outcome through the use of fibrin glue [for a review see Tseng et al, 2005].

A. For Corneal Surface Reconstruction

1) Corneal Ulcers with Different Depths Including Perforation

Corneal ulcers are serious and urgent clinical problems that can be complicated by microbial infections and threaten patient's vision. Corneal ulcers can be caused by various insults, e.g., exogenously from chemical burns, infection, radiation, or surgeries, while endogenously from aging, diabetes mellitus, viral (herpes) infection, and autoimmune disorders. Corneal ulcers are relatively uncommon and require immediate attention. When medical treatments fail and the ulceration persists (e.g., more than 3 weeks), conventional surgical treatments are usually indicated and include lamellar or full-thickness corneal transplantation (transplantation of allogenic cornea), tarsorrhaphy (closure of patient's lids) and conjunctival flap (transferring patient's own conjunctiva to cover the diseased cornea). Amniotic membrane transplantation offers the following advantages, e.g., avoidance of potential complications of corneal transplantation including allograft rejection, feasibility in lieu of cornea tissues in places where there is a shortage of cornea tissues, preservation of patient's cosmetic appearance without lid closure or covering of the cornea with a vascularized conjunctival tissue.

A total of 10 studies have been reported. All 10 reports noted that ocular surface inflammation is markedly reduced following transplantation (nearly in all cases), and the defect covered by amniotic membrane heals rapidly (1 to 4 weeks). The overall success of healing the ulcer without recurrence ranges from 67% to 91%, with an average of 78.6% (103/131 eyes). Variable success rates are attributed to differences in the underlying etiology, depths of ulceration, and accompanied treatments. Some patients regained more vision after transplantation.

Lee and Tseng[101] first reported that amniotic membrane transplantation results in rapid healing in 3.9 ± 2.3 weeks, complete healing without recurrence in 10/11 consecutive eyes for a follow up of 9.0 ± 5.9 months. One failure case had severe rheumatoid arthritis. Kruse et al[102] reported the use of multiple layers of amniotic membrane to fill in ulcers with significant depths and noted rapid healing in 4 weeks, remained stable in 9/11 eyes for 1 year. Two patients recurred due to severe neurotrophic keratopathy (loss of corneal innervation). Azuara-Blanco and Dua[103] reported complete healing in 4/5 eyes. Chen et al[104] reported rapid healing in 16.6 ± 9.0 days in 14/16 eyes of severe neurotrophic ulcers with varying depths for a follow up of 18.8 ± 13.0 months. The four failure cases (presumably due to more severe involvement) required tarsorrhaphy and corneal transplantation to heal the refractory ulceration. Gabric et al[105] reported success of healing corneal ulcers in 8/12 eyes in 1-3 weeks. Hanada et al[106] reported complete healing in 16.5 ± 8.0 days in 8/11 eyes using multiple layers of amniotic membrane to treat deep corneal ulcers with descemetocele (i.e., total ulceration up to the descemet membrane) (n=5), with additional scleral ulceration (n=2), and corneal perforation (n=4). One failure eye had limbal stem cell deficiency due to chemical burns and two eyes had severe rheumatoid arthritis. Letko et al[107] reported successful healing of 21/30 eyes with persistent corneal epithelial defects which were refractory to contact lens and tarsorrhaphy with an average healing time of 25.5 days

after surgery. Su and Lin[108] reported one case report of successful treatment of corneal perforation using amniotic membrane and tissue adhesive. Heinz et al, [97] reported the successful epithelial and stromal healing in one patient with severe Graves ophthalmopathy with a corneal ulcer refractive to topical treatment and orbital decompression surgery. Rodriguez et al reported successful treatment of corneal ulcers of different sizes in 11/15 eyes (73%), by using multilayer amniotic membrane transplantation. Three of the four unsuccessful treatments were of perforations 3 mm or more in diameter. The authors concluded that multilayer AMT is effective for corneal perforations with diameter less than 1.5 mm and suggested that it may be a good alternative to penetrating keratoplasty, especially in acute cases in which graft rejection risk is high. [109] . [110] reported successful management of corneal ulcers and perforations in 27/33 eyes. Perforated ulcers up to 3 mm in diameter (n=14) received fibrin glue and amniotic membrane. Ulcers healed in a mean time of 3.6 ± 1.6 weeks. Failure was noted in 6 eyes with severe neurotrophic keratitis, Stevens-Johnson syndrome, ocular cicatricial pemphigoid and Acanthamoeba keratitis.

2) Symptomatic Bullous Keratopathy

Bullous keratopathy, i.e., corneal edema, is a disorder caused by corneal endothelial decompensation due to degeneration (Fuch's endothelial dystrophy), surgical trauma, intractable glaucoma, or previous corneal graft failure. Patients with bullous keratopathy complain of ocular pain and loss of vision. For those patients with potenital vision, corneal transplantation is the treatment of choice. However, for those who do not have a visual potential, relief of pain will rely on several different surgical treatments including cauterization, anterior stromal puncture, excimer laser photoablation, and conjunctival flap. Without treatment, the disease showed a progressive deterioration with persistent ocular discomfort.

Because amniotic membrane can be an ideal substrate to improve the corneal stroma, Pires et al[111] reported an overall success of 90% (43/48 eyes) of achieving a stable surface and pain relief for the follow up period of 33.8 weeks (3 – 96 weeks). Among the five failure cases, 3 received repeated amniotic membrane transplantation and one received conjunctival flap for pain relief. The amniotic membrane covered corneal surface healed with 3 weeks. Only 4 (8%) showed recurrent surface breakdown. The reconstructed corneal surface showed reduced inflammation. Espana et al [112] reported 88% pain relief in 18 eyes (18 patients) during a mean follow up of 25.1 months (12 to 45 months). One eye had evisceration for persistent pain 10 months postoperatively. Dua and Gomes [10] have used the amniotic membrane in 16 patients with symptomatic bullous keratopathy; finding rapid epithelialization of the membrane, usually within 7 days. Post-operative pain was significantly less than pre-operative but long term follow-up results are awaited.

3) Band Keratopathy

Band keratopathy, i.e., calcium deposit on the corneal surface, occurs in a number of corneal diseases characterized by chronic inflammation and sometimes bullous

keratopathy. Patients with band keratopathy complain of ocular irritation and experience corneal surface erosion and breakdown, leading to a threat of possible microbial infection. Conventional treatments include chelation by EDTA and superficial keratectomy, i.e., removal of the superficial calcium deposit and the corneal stromal tissue. Without treatment, band keratopathy does not show any remission and instead has a slowly progressive clinical course. Anderson et al[113] reported 14/15 eyes (93%) of success of using amniotic membrane transplantation to relieve patient's pain, and achieved 15/16 eyes (94%) epithelialization in an average of 15.2 days during the mean follow up period of 14.6 months. Vision improved in 5/9 sighted eyes (44%).

4) Partial Limbal Stem Cell Deficiency

The epithelial stem cells are located exclusively at the limbus, i.e., the anatomic junction between the cornea and the conjunctiva. Limbal epithelial stem cells are responsible for regeneration of the corneal epithelium, which has a rapid turnover rate. Destructive loss of the limbal epithelial stem cells and/or dysfunction of the limbal stroma will lead to limbal stem cell deficiency, which is characterized by conjunctivalization of the cornea, i.e., the conjunctival epithelium migrates to cover the corneal surface, which is accompanied by vascularization, destruction of the basement membrane, chronic inflammation, and scarring of the cornea [for cytological evidence see[114]]. Limbal stem cell deficiency can be caused by a number of corneal diseases such as chemical and thermal burns, Stevens-Johnson syndrome, ocular pemphigoid, severe microbial infections, radiation keratopathy, aniridia, etc. Patients suffer from limbal stem cell deficiency complain of severe photophobia (light sensitivity) and severe loss of vision. Without treatment, limbal stem cell deficiency is a progressively worsened with time. Conventional corneal transplantation invariably fails, as no stem cells are transplanted, and frequently rejected due to corneal vascularization and inflammation. New surgical strategy resorts to autologous or allogeneic transplantation of limbal epithelial stem cells.[115] Amniotic membrane used as a surgical graft was popularized by Kim and Tseng[116, 117] in a rabbit model of limbal stem cell deficiency. They reported a surprising 40% success in 13 eyes, with recovery of a normal corneal epithelial phenotype as compared to 100% failure in 10 control eyes, which showed a conjunctival phenotype.

Tseng et al[118] reported a success of 100% in 8/8 eyes with *partial* limbal stem cell deficiency, i.e., partial loss of host limbal stem cells, suggesting that amniotic membrane transplantation alone is sufficient to restore the corneal surface in this entity without the use of limbal stem cell transplantation. When the follow-up period was extended for an average of 25.8 months, Anderson et al[119] noted that there was still an overall success of 93% of 14 sighted eyes with *partial* limbal stem cell deficiency and 86% of 17 such eyes with reduction of photophobia and pain. More recently, Sangwan et al [120] studied the efficacy of amniotic membrane transplantation in reconstructing the corneal epithelial surface and visual rehabilitation in 4 patients with partial limbal stem cell deficiency. All eyes exhibited stable corneal epithelial surface by an average of 7 weeks postoperatively and best corrected visual acuity improved an average of 4.5 lines on Snellen visual acuity charts.

C. For Conjunctival Surface Reconstruction

When a large conjunctival lesion is surgically removed, the conjunctival defect is normally healed by the surrounding conjunctiva with granulation and scarring, which may lead to disfiguring and motility restriction of the extraocular muscles or the lid blinking. To avoid such potential problems, conjunctival autograft from the same eye or the fellow eye is frequently used. However, some patients might not have healthy conjunctival tissue to spare and further removal of the uninvolved conjunctiva might put the patient at additional risks. De Rotth[121] in 1940 first used *live* fetal membrane (i.e., amnion plus chorion) for conjunctival surface reconstruction during symblepharon lysis (i.e., to release the adhesion between the bulbar and the tarsal conjunctival surface). Probably due to the inclusion of chorion and his use of live tissue, the success rate of 1/6 eyes was not impressive.

Based on the aforementioned improved method of preparation and preservation, amniotic membrane transplantation has been used for conjunctival surface reconstruction. In general, studies showed that the defect covered by amniotic membrane heals rapidly, and the resultant surface is less inflamed with minimal scarring in those cases with a success.

1) Conjunctivochalasis

Conjunctivochalasis is defined as a conjunctival redundancy, frequently seen in the older age group as an elevation of the bulbar conjunctiva lying along the lower lid margin [122] ; but not restricted to it, as described by Di Pascuale et al [123] . Conjunctivochalasis can cause a spectrum of symptoms, ranging from aggravation of a dry eye at the mild stage, to disturbance of tear outflow at the moderate stage, and exposure problems at the severe stage. For symptomatic patients, topical lubricants can be tried, but they are frequently unsuccessful, and surgical excision may be required. Meller et al[124] reported successful reconstruction of conjunctival surface following the removal of conjunctivochalasis, i.e., redundant conjunctiva, in 46/47 consecutive eyes (98%) with resolution of ocular irritation. Complications included focal inflammation of the host conjunctiva adjacent to the amniotic membrane graft (6 eyes), scar formation (5 eyes), and suture-induced granuloma (1 eye). Georgiadis et al [125] reported resolution of symptoms in 12 patients with chronic epiphora caused by conjunctivochalasis, after removal of the excess of conjunctiva followed by amniotic membrane transplantation; during a mean follow-up of 8 months.

2) Tumors

Amniotic membrane transplantation has been used in for conjunctival surface reconstruction when a large lesion is removed. In general, studies showed that the defect covered by amniotic membrane heals rapidly, and the resultant surface is less inflamed with minimal scarring in those cases with a success.

Tseng et al[126] first reported successful reconstruction in 11/17 eyes (65%) with removal of melanoma (n=1), melanosis (n=1), conjunctivochalasis (n=1), conjunctival intraepithelial neoplasia (n=3), conjunctival scarring without symblepharon (n=3), and conjunctival scarring with symblepharon (n= 8) in a follow up period of 10.9 ± 9.1 months. The defect covered by amniotic membrane healed in 3 weeks. In three of such patients, impression cytology confirmed the restoration of a normal conjunctival epithelial phenotype with goblet cells.[127] Paridaens et al[128] reported successful reconstruction of conjunctival surface in 3/4 eyes (75%) following the removal of malignant melanoma and primary acquired melanosis with atypia with amniotic membrane transplantation. Chen et al [129] referred amniotic membrane transplantation as a very effective method to repair wound after conjunctival tumor removal in 26 patients (26 eyes) including 9 eyes with malignant tumors (conjunctival melanoma, corneal and conjunctival squamous cell carcinoma and conjunctival lymphoma), 17 eyes with benign tumors (conjunctival papilloma, conjunctival nevus, etc.). Dalla et al [130] reported successful reconstruction of conjunctival surface in 4/4 patients with diffuse conjunctival melanoma after a minimum follow-up of 48 months.

3) Glaucoma Surgery / Leaking Bleb

The anti-inflammatory and anti-scarring effects of amniotic membrane prompted Barton et al[131] to investigate in rabbits the efficacy of maintaining glaucoma filtration procedure. Fujishima et al[132] reported the success of maintaining trabeculectomy using amniotic membrane transplantation in conjunction with 0.4 mg/ml of mitomycin C application. Budenz et al[133] reported in a randomized and prospective clinical study that amniotic membrane grafts achieved the same pressure-lowering effect in repairing leaking glaucoma filtering blebs as conjunctival advancement surgery in a mean follow up of 19 months. However, the cumulative survival rate for amniotic membrane graft was 81% at 6 months and 46% in 2 years compared to 100% for conjunctival advancement procedure. Gomez and Dua [10] have reported very different results from one and other regarding the use of amniotic membrane in glaucoma surgery. While Gomez using the amniotic membrane transplantation in 4 patients (4 eyes) presenting post-trabeculectomy with mitomycin C complications (leaking blebs), noticed completed resolution of the problem. Dua used it in 2 patients to cover the exposed pericardial patch, which was used to cover the tube of a valve implant and in both patients the amniotic membrane underwent necrosis, and was replaced with an autologous conjunctival patch with good results.

4) Scleral Melt

Rodriquez-Ares et al[134] reported a single case of successful reconstruction of conjunctival surface and sclera in a patient with Marfan's syndrome with extensive scleral defect.

5) Pterygium

Pterygium is a common eye disease caused by chronic exposure to ultraviolet light. Pterygium is a disease characterized by progressive fibrovascular proliferation of the stroma and the dysfunction of the adjacent limbal epithelial stem cells. The mainstay of therapy remains to be surgical. Following the removal of pterygium by a bare sclera technique, the denuded conjunctival surface is left either uncovered or covered with a graft. For the former without a graft, adjunctive therapies such as topical application of mitomycin C or external beta irradiation is needed to reduce the recurrence rate, which is otherwise quite high. These two adjunctive therapies are associated with such complications as scleral melt and microbial infections. For the latter with a graft, the conventional graft used is conjunctival autograft, where a part of free conjunctival tissue is taken from the same eye or the uninvolved fellow eye and used to cover the conjunctival defect. The use of conjunctival autograft is however limited in patients with more than one pterygium in the eye, or in patients with recurrent pterygium after several excisions or following previous conjunctival autograft, or in patients with glaucoma where the donor site is reserved for the prospective filtering procedure. For all of these clinical situations, amniotic membrane may be used as an alternative graft.

The following 8 studies used amniotic membrane transplantation for conjunctival surface reconstruction following removal of primary or recurrent pterygium. For primary pterygium, Prabhasawat et al[135] first compared a prospective study using amniotic membrane grafts (n=54) to a retrospective study using conjunctival autografts (n=122) in both primary and recurrent pterygium. They noted that the recurrence rate is 10.9% using amniotic membrane grafts, which is still higher than 2.6% of conjunctival grafts. Nevertheless, both results of amniotic membrane grafts and conjunctival autografts are significantly better than the primary closure (n=20), which resulted in 45% high recurrence rate for primary pterygium. Subsequently, Solomon et al[136] reported that by incorporating a larger removal of subconjunctival fibrous tissue and injection of long-acting steroid, amniotic membrane grafts achieved a lower recurrence rate of 3.0%, compatible with 2.6% of conjunctival autografts published by Prabhasawat et al.[135] Kim et al[137] reported a recurrence rate of 18% in 11 primary pterygium. Ma et al[138] reported 3.7% recurrence rate in 80 eyes using amniotic membrane grafts, which is compatible with 5.4% of 56 eyes with conjunctival autografts, and 3.7% of 54 eyes with topical mitomycin C, an anti-metabolite that inhibits cell proliferation, in primary pterygium. Ang et al [129, 139] compared the use of autologous cultivated conjunctival transplantation and conventional amniotic membrane transplantation for the treatment of primary pterygium in 40 patients. Patients were divided in 2 groups, A and B respectively. Complete epithelialization was achieved 5 days after surgery in group A and approx 3 weeks after the surgery in group B. After a mean follow-up of 14.1 months (12 to 25 months) the recurrence rate was 22.7% in group A and 25.0% in group B. They only noticed one ocular complication, affecting a patient in group B who developed scleral necrosis associated with a persistent epithelial defect.

Recurrent pterygium represents a more aggressive disease. In the study conducted by Prabhasawat et al[135] , the recurrence rate is 37.5% for recurrent pterygium, which is much higher than 9.5% using conjunctival autografts for recurrent pterygium. Gabric et a[105] reported a 30% recurrence in 10 eyes with recurrent pterygium using amniotic

membrane grafts. Subsequently, Solomon et a[136] reported that by incorporating a larger removal of subconjunctival fibrous tissue and injection of long-acting steroid, amniotic membrane grafts achieved a lower recurrence rate of 9.5%, which was compatible with 9.5% using conjunctival autografts for recurrent pterygium reported by Prabhasawat et al.[135] As recurrent pterygium frequently receives more than one surgery and there is a great deal of shortage of normal conjunctival adjacent to the diseased area, it is theoretically advantageous to add a conjunctival autograft, which will bring in some healthy conjunctival epithelial stem cells. The size of this conjunctival autograft is much smaller than that normally used without amniotic membrane transplantation. Using this new approach, Kim et al[137] reported that no recurrence in 9 eyes with recurrent pterygium, and Shimazaki et al[140] reported no recurrence in 4 eyes with recurrent pterygium Kawasaki et al [141] reported a 12% recurrence in 26 eyes with recurrent pterygium. The mean follow-up time was 17.3 +/- 9.3 months. All patients were operated on with an extensive excision of the subconjunctival fibrous tissues followed by application of mitomycin C (0.04%, 3-5 min) during the operation. Amniotic membrane transplantation was performed with implantation of conjunctival autograft in cases with large excision. Ma et al [142] compared the efficacy and safety of amniotic membrane graft alone (n=48) and combined with intraoperative mitomycin C (0.025%, 3 min) (n=46) after excision of recurrent pterygium, in 95 patients. No significant difference in the recurrence rate between the two groups was found after a mean follow-up of 12 months. In the AMT group, 6 conjuctival (12.5%) and 6 corneal (12.5%) recurrences developed. In the AMT-mitomycin group 4 conjunctival (8.5%) and 6 corneal (12.5%) recurrences developed.

6) Symblepharon and Fornix Reconstruction

For cases of symblepharon; i.e,, scar in the conjunctiva causing motility restriction, the main goal is to achieve a deep fornix and lack of motility restriction. Azuara-Blanco and Dua[103] reported that a successful reconstruction of one case with symblepharon. Gabric et al[105] reported success of conjunctival reconstruction in 5/6 eyes with conjunctival scarring. Honavar et al[143] reported successful fornix reconstruction in 9/10 eyes with symblepharon in patients with Stevens-Johnson syndrome in a mean follow up period of 13.5 ± 3.8 months. The complete healing took 1 to 6 weeks. Solomon et al[144] reported complete fornix reconstruction in 12/17 eyes (70.6%) of 15 patients with symblepharon due to a variety of ocular disorders, after a mean follow-up of 37 +/- 24 months (9-84 months). In eyes that demonstrated partial success of failure, the underlying etiology was either an autoimmune disorder or a recurrent pterygium. Barabino et al [145] reported the improvement of the ocular surface condition, with reacquired fornix depth, and reduced inflammation in one patient with late stage ocular cicatricial penphigoid. Later on, Barabino et al [146] reported successful symblepharon lysis in 5/9 eyes (9 patients) with advanced ocular cicatricial penphigoid, during a mean follow-up of 48 weeks (28-96 weeks). Katircioglu et al [147] reported successful fornix reconstruction in 5/6 eyes in patients with symblepharon due to chemical burns in a mean follow-up of 10 +/- 7.37 months (4-24 months). Jain et al [148] noticed the effectiveness of the AMT in the surgical treatment of symblepharon in 12/20 eyes of 18 patients. Zhou et al [149] reported successful fornix reconstruction in 31/55 eyes after a mean follow-up of 27 +/- 2.6 months (26-30 months). Tseng et al[150] used intraoperative application of

mitomycin C (0.04% for 5 minutes) to reduce chronic conjunctival inflammation, helping amniotic membrane to restore a deep fornix after symblepharon lysis in 18 eyes (16 patients) suffering from various diseases. They reported successful fornix reconstruction in 15/18 eyes after a mean follow-up of 14.16 ± 5.2 months. Nava-Castaneda et al [149, 151] reported better conjuctival fornix reconstruction with the simultaneous combination of AM and mitomycin C than with the use of AM alone.

D. For Both Corneal and Conjunctival Surface Reconstruction

Amniotic membrane can also be used for treating cicatrizing conditions involving the both the corneal and conjunctival surfaces. For example, in eyes with chemical burns involving the conjunctival and sectorial corneal surface with pannus, amniotic membrane can be used for symblepharon lysis, fornix reconstruction of the conjunctival region while at the same time, part of amniotic membrane can be extended to cover the corneal surface where the pannus is removed by superficial keratectomy.

E. In Conjunction with Other Surgical Procedures

As mentioned before, amniotic membrane transplantation alone is sufficient to restore corneal surfaces that have partial limbal stem cell deficiency (i.e., with some remaining healthy stem cells) in human patients.[118, 119] Nevertheless when used in conjunction with other surgical procedures to provide some epithelial stem cells, amniotic membrane transplantation is effective to restore the ocular surface in patients with severe ocular disorders.

A total of 7 studies have been reported using amniotic membrane with or without limbal stem cell transplantation for reconstructing the corneal surface with limbal stem cell deficiency. The overall success depends on the severity of the limbal stem cell deficiency, i.e., partial versus total, accompanied corneal diseases, and severity of the ocular surface illness, such as dry eye. For *partial* limbal stem cell deficiency, amniotic membrane transplantation is a superior alternative as it alone without limbal stem cell transplantation is sufficient to restore the corneal surface and improve the vision in a majority of the patients. For unilateral *total* limbal stem cell deficiency, autologous limbal stem cell transplantation can be performed in conjunction with amniotic membrane transplantation.[152] Thus, amniotic membrane transplantation augments the success of limbal stem cell transplantation. The overall success of the procedure is very high. For bilateral *total* limbal stem cell deficiency, allogeneic limbal stem cell transplantation is needed to restore such damaged cornea [90, 149] . The overall success is further influenced by the survival of this limbal allograft (see below for more details).

1) With Conjunctival Limbal Autograft or Allograft

Shimazaki et al[85] reported successful reconstruction of corneal surface damaged by chemical and thermal burns in 7/7 eyes (100%) using amniotic membrane

transplantation and limbal stem cell transplantation of an autologous (n=4) and allogeneic (n=2) source in a mean follow up period of 53.3 weeks. Later on, Shimazaki et al [153] reported successful treatment in 23/27 eyes (85.2%) with recurrent pterygium by using amniotic membrane transplantation combined either with limbal autograft transplantation (n=15) or conjuctival autograft transplantation (n=12), after a mean follow-up of 67.0 weeks. Gomes et al [154] reported satisfactory ocular surface reconstruction in 15/20 eyes (75%) with chemical burns with limbal stem cell deficiency, using amniotic membrane transplantation with or without limbal transplantation from either autologous or allogenic source, after a mean follow-up of 19 months (8-27months). Success was observed in all cases of partial limbal stem cell defiency and in 68.75% (11 eyes) of cases of total limbal stem cell deficiency. Meallet et al[155] reported successful corneal surface reconstruction in 5 eyes (5 patients) with total limbal stem cell deficiency, using conjunctival limbal autograft combined with amniotic membrane transplantation on both the donor and recipient eyes. During the mean follow-up of 22 months (11-48 monyhs) all eyes experienced symptomatic relief. All recipient eyes had a mean improvement in visual acuity of nine lines (range 7-12). The donor eyes showed rapid healing and restoration of the normal limbal landmark. Gomes et al [156] reported only 20% (2/10 eyes) success in ocular surface reconstruction in 10 patients with total limbal stem cells and conjunctival deficiency secondary to Stevens-Johnsons syndrome, after a mean follow-up of 16.7 months. Patients underwent excision of cicatricial tissue followed by amniotic membrane and living related corneal/limbal conjunctival transplantation. Yao et al [157] reported the successful approach for treating multirecurrent pterygia with severe symblepharon by combining intraoperative mitomycin C, amniotic membrane and limbal conjuctival autograft in 6/7 eyes (7 patients), for a mean follow-up of 22.4 ± 6.1 months. Miyai et al [153, 158] describe the use of limbal allograft, preserved amniotic membrane transplantation, and intraoperative mitomycin C, as a safe and effective procedure for treating recurrent pterygium. They evaluate 12 eyes of 11 patients. After a mean follow-up period of 21.6 ± 5.6 months, there was no pterygium recurrence, symblepharon recurred in 3 eyes and diplopia in 2 eyes. Santos et al [153, 159] reported the usefulness of the conjunctival limbal grafts associated with AMT for restoring corneal epithelial phenotype in 33 eyes of 31 patients with total LSCD. They also describe dry eye as the most important prognostic parameter. However, they found that the cumulative survival declines substantially over a 2-year period.

2) With Keratolimbal Allograft

Tsubota et al reported successful reconstruction of the corneal surface in 12/14 eyes (86%) with *total* limbal stem cell deficiency due to severe and advanced ocular pemphigoid and Stevens-Johnson syndrome using amniotic membrane transplantation in conjunction with keratolimbal allograft in a mean follow up period of 143 days. In childrens with Stevens-Johnson syndrome Tsubota et al[160] reported successful reconstruction in 3/5 eyes using amniotic membrane transplantation and keratolimbal allograft. Tseng et al[118] confirmed that amniotic membrane transplantation needed to be combined with keratolimbal allograft when there was a total loss of limbal epithelial stem cells. In a total of 21 eyes (n=7 without additional corneal transplantation; n=14 with

additional corneal transplantation), they noted successful corneal surface reconstruction of 71% and 79%, respectively, in a mean follow-up period of 15.4 months. When the follow-up period was extended to an average of 1163 days (over 3 years), Tsubota et al[161] noted that in their large series of 43 eyes with *total* limbal stem cell deficiency, the overall success rate is reduced to 51% due to progressive allograft rejection of the keratolimbal transplant despite amniotic membrane transplantation. Another major limiting factor to the success of such corneal surface reconstruction is the presence of severe aqueous tear deficiency, i.e., dry eye.[162] Espana et al [163] did a phenotypic study of a case receiving a keratolimbal allograft and amniotic membrane for a total stem cell deficiency, finding a normal corneal epithelial phenotype with normal base membrane complexes. Jurowski et al [164] reported good results in 3 eyes (2 patients) with chemical (acid) burns. Shimazaki et al [165] described their findings in 32 eyes (32 patients) with chemical burns associated with total limbal dysfunction. Patients were treated by amniotic membrane transplantation combined either with conjunctivolimbal autograft transplantation (n=11) or keratolimbal allograft transplantation (n=21). At final examination, 17 eyes (53.1%) showed stable corneal epithelialization. The autograft group showed significantly better results than the allograft group in both corneal epithelization and clear cornea.

IV. Complications

There have not been any report showing microbial infections directly linked with amniotic membrane transplantation. Kim et al[137] reported such complications as submembrane hemorrhage (3/25 eyes, 12%) and early detachment of the membrane (1/25, 4%). The former is obviously related to the surgery and not amniotic membrane. Gabler and Lohmann[166] reported a case who developed sterile hypopyon (inflammation inside the anterior chamber) following repeated transplantation of amniotic membrane. They attributed this complication to immunologic, toxic or hypersensitive effect of the membrane. No similar complication has been reported by others.

Marangon et al [167] reported the incidence and characteristics of post-AMT infections of a total of 326 patients undergoing AMT from January 1994 to February 2001 at the Bascom Palmer Eye Institute. They subdivided these patients in 2 groups related to the submission or not of the AMT storage media for culture under an institutional Review Board-approval protocol. 11 culture positive infections (3.4%) were identified. 7 (9.2%) were from the first group and 4 (1.6%) were from the second group. All infections occurring within 1 month after AMT (n=4) were exclusively from the first group. All AM storage media from the second group were culture negative. Gram-positive organisms were the most frequent isolated (64%). Finally they concluded that AMT is a safe method for ocular surface reconstruction with a very low rate of microbial infections, especially if AM is prepared according to Good Tissue Banking Practice set forth by FDA.

Gomez [10] observed a granulomatous reaction, unrelated to sutures, a few days after ocular surface reconstruction combined with a living related conjunctival limbal allograft in a Stevens-Johnson syndrome patient.

V. Other Issues

Since amniotic membrane was introduced for the management of ocular surface disorders, it has been harvested, prepared in many different ways. The earliest report in ophthalmology, dating 1940 is by De Roth, who used fresh membrane, including both, chorion and amnion, as a dressing in patients with conjuctival defects. Laverly and Sorsby in 1946 and Symmons in 1947 used an "amnioplastin graft", a chemically processed dry amniotic membrane, in the treatment of patients with chemical burns. In 1993 Batle and Perdomo reported the use of alcohol-preserved AM (Russian Method). Over the years the tissue-processing techniques have evolved; thus in 1995, Kim and Tseng reported the use of cryopreserved AM in a rabbit model. Since then it has been a great development of the amniotic membrane in ophthalmic surgery. [10, 11, 15] Although a freeze-dried AM is used, it has not been approved by FDA as a surgical graft (see FDA website: http://www.fda.gov/cber/compl/ambio062305.htm. Finally, it should be noted that fresh amniotic membrane is still commonly used outside the US. [168-172] . The use of fresh amniotic membrane is not approved by FDA in USA.

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