

# Literature Summary on Uses of Amniotic Membrane in Ocular Surface Reconstruction

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## **I. Use of Amniotic Membrane in Ocular Surface Reconstruction**

Amniotic membrane, the innermost layer of fetal (or placental) membrane, consists of a thick basement membrane and an avascular stroma. Its function is to protect the fetus from unwanted maternal insults during development. It has been commonly recognized that the incision made via the skin of the fetus during fetal surgery performed in the third trimester does not bear any scarring after birth. The phenomenon of “scar-less fetal wound healing” remains to be elucidated. It is not clear if amniotic membrane carries the same feature as the fetal tissue.

According to the reported clinical uses of amniotic membrane transplantation for ocular surface reconstruction, the reconstructed corneal or conjunctival surfaces show rapid epithelialization (i.e., covering of the epithelial cells onto the denuded amniotic membrane) and reduced inflammation and scarring. In other words, the membrane facilitates healing and regeneration of cells with a minimum of inflammation and scarring.

## **II. Potential Action Mechanisms of Amniotic Membrane in Ocular Surface Reconstruction**

There are a number of action mechanisms that may explain the effectiveness of amniotic membrane used in ocular surface reconstruction. They are:

- The amniotic membrane's basement membrane contains type IV collagen, laminin 1, laminin 5, fibronectin, and collagen VII. One component, the collagen IV subchain, is identical to that of the conjunctiva,<sup>1</sup> and laminins are particularly effective in facilitating corneal epithelial cell adhesion.<sup>2</sup> In general the basement membrane side of amniotic tissue is an ideal substrate for supporting the growth of epithelial progenitor cells. Epithelial cells, including stem cells, anchor to the basement membrane. In general, 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.<sup>3</sup> Amniotic membrane, like many other tissues in the body,

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1 Fukuda K, Chikama T, Nakamura M, Nishida T. Differential distribution of subchains of the basement membrane components type IV collagen and laminin among the amniotic membrane, cornea, and conjunctiva. *Cornea*. 1999; 18:73-79.

2 Kurpakus MA, Daneshvar C, Davenport J, Kim A. Human corneal epithelial cell adhesion to laminins. *Curr Eye Res*. 1999;19:106-114.

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

Boudreau N, Werb Z, Bissell MJ. Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. *Proc Natl Acad Sci USA*. 1996;93:3500-3513.

Weaver VM, Bissell M. Functional culture models to study mechanisms governing apoptosis in normal and malignant mammary epithelial cells. *J Mammary Gland Bio Neoplasia*. 1999;4:193-201.

contains several mitogenic growth factors.<sup>4</sup> Collectively, these data may explain why amniotic membrane maintains the normal epithelial phenotype of human conjunctival<sup>5</sup> and corneal<sup>6</sup> epithelial cells in culture.

- The amniotic membrane's stroma contains growth factors,<sup>7</sup> anti-angiogenic and anti-inflammatory proteins,<sup>8</sup> and natural inhibitors to various proteases.<sup>9</sup> Expression of IL-1 $\alpha$  and IL-1 $\beta$  is markedly suppressed when human limbal epithelial cells are cultured on the amniotic membrane stromal matrix, even when challenged by lipopolysaccharide.<sup>10</sup> 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.<sup>11</sup> This finding was supported in human patients with acute burns where CD20+ cells are trapped by amniotic membrane and exhibited apoptosis.<sup>12</sup> When rabbit's corneas are injured by alkali, amniotic membrane transplantation reduces this acute and severe inflammation as

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- 4 Koizumi N, Inatomi T, Sotozono C, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res.* 2000;20:173-177.
- 5 Cho B-J, Djalilian AR, Obritsch WF, Matteson DM, Chan CC, Holland EJ. Conjunctival epithelial cells cultured on human amniotic membrane fail to transdifferentiate into corneal epithelial-type cells. *Cornea.* 1999;18:216-224.
- Meller D, Tseng SCG. Conjunctival epithelial cell differentiation on amniotic membrane. *Invest Ophthalmol Vis Sci.* 1999;40:878-886.
- 6 Koizumi N, Inatomi T, Quantock AJ, Fullwood NJ, Dota A, Kinoshita S. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. *Cornea.* 2000;19:65-71.
- Koizumi N, Fullwood NJ, Bairaktaris G, Inatomi T, Kinoshita S, Quantock AJ. Cultivation of corneal epithelial cells on intact and denuded human amniotic membrane. *Invest Ophthalmol Vis Sci.* 2000;41:2506-2513.
- 7 Koizumi N, Inatomi T, Sotozono C, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res.* 2000;20:173-177.
- 8 Hao Y, Ma DH-K, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea.* 2000;19:348-352.
- 9 Na BK, Hwang JH, Kim JC, Shin EJ, Kim JS, Jeong JM, Song CY. Analysis of human amniotic membrane components as proteinase inhibitors for development of therapeutic agent of recalcitrant keratitis. *Trophoblast Res.* 1999;13:459-466.
- 10 Solomon A, Rosenblatt M, Monroy DC, Ji Z, Pflugfelder SC, Tseng SCG. Suppression of Interleukin-1 $\alpha$  and Interleukin-1 $\beta$  in the human corneal epithelial cells cultured on the amniotic membrane matrix. *Br J Ophthalmol.* 2001;85:444-449.
- 11 Park WC, Tseng SCG. Muculation of acute inflammation and keratocyte death by suturing, blood and amniotic membrane in PRK. *Invest Ophthalmol Vis Sci.* 2000;41:2906-2914.
- Wang MX, Gray TB, Parks WC, Prabhasawat P, Culbertson WW, Forster RK, Hanna K, Tseng SCG. Corneal haze and apoptosis is reduced by amniotic membrane matrix in excimer laser photoablation in rabbits. *J Cat Refract Surg.* 2001;27:310-319.
- 12 Shimmura S, Shimazaki J, Ohashi Y, Tsubota K. Antiinflammatory effects of amniotic membrane transplantation in ocular surface disorders. *Cornea.* 2001;20:408-413.

evidenced by less amount of infiltration of polymorphonuclear neutrophils.<sup>13</sup> 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. The infiltration of polymorphonuclear neutrophils, lymphocytes, and macrophages is all reduced.<sup>14</sup> Collectively, the above findings provide evidence to support the anti-inflammatory effect of amniotic membrane transplantation.

- The amniotic membrane stroma suppresses TGF- $\beta$  signaling and myofibroblast differentiation for cultured human corneal fibroblasts and limbal fibroblasts<sup>15</sup> and cultured human conjunctival fibroblasts and pterygium body fibroblasts.<sup>16</sup> Therefore, besides the aforementioned anti-inflammatory effect, which may indirectly yield an anti-scarring effect, there is a strong evidence that amniotic membrane has a direct anti-scarring effect. Collectively, these actions explain why amniotic membrane transplantation into the corneal stromal pocket reduces the myofibroblast differentiation elicited by invading epithelial cells in a rabbit model and in a tissue culture model of collagen gel contraction.<sup>17</sup> They also explain why corneal scarring (also termed haze) is reduced in excimer laser-induced keratectomy in rabbits by amniotic membrane transplantation.<sup>18</sup> Such a reduction of corneal haze has been explained by the reduction of unwanted keratocyte apoptosis (cell death of the normal healthy corneal stromal fibroblasts)<sup>19</sup> and the reduction of newly synthesized extracellular matrix (i.e., scarred

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- 13 Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits protease activity on wound healing following acute corneal alkali burns. *Exp Eye Res.* 1998;70:329-337.
- 14 Nobe JR, Moura BT, Robin JB, Smith RE. Results of penetrating keratoplasty for the treatment of corneal perforations. *Arch Ophthalmol.* 1990;108:939-941.
- 15 Tseng SCG, Li D-Q, Ma X. Suppression of Transforming Growth Factor isoforms, TGF- $\beta$  receptor II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. *J Cell Physiol.* 1999;179:325-335.
- 16 Lee S-B, Li D-Q, Tan DTH, Meller D, Tseng SCG. Suppression of TGF- $\beta$  signaling in both normal conjunctival fibroblasts and pterygial body fibroblasts by amniotic membrane. *Curr Eye Res.* 2000;20:325-334.
- 17 Choi TH, Tseng SCG. *In vivo* and *in vitro* demonstration of epithelial cell-induced myofibroblast differentiation of keratocytes and an inhibitory effect by amniotic membrane. *Cornea.* 2001;20:197-204.
- 18 Wang MX, Gray TB, Parks WC, Prabhasawat P, Culbertson WW, Forster RK, Hanna K, Tseng SCG. Corneal haze and apoptosis is reduced by amniotic membrane matrix in excimer laser photoablation in rabbits. *J Cat Refract Surg.* 2001;27:310-319.
- Choi YS, Kim JY, Wee WR, Lee JH. Effect of the application of human amniotic membrane on rabbit corneal wound healing after excimer laser photorefractive keratectomy. *Cornea.* 1998;17:389-395.
- 19 Park WC, Tseng SCG. Modulation of acute inflammation and keratocyte death by suturing, blood and amniotic membrane in PRK. *Invest Ophthalmol Vis Sci.* 2000;41:2906-2914.
- Wang MX, Gray TB, Parks WC, Prabhasawat P, Culbertson WW, Forster RK, Hanna K, Tseng SCG. Corneal haze and apoptosis is reduced by amniotic membrane matrix in excimer laser photoablation in rabbits. *J Cat Refract Surg.* 2001;27:310-319.

connective tissue).<sup>20</sup> Collectively, these experimental data support the notion that amniotic membrane transplantation reduce scar formation, i.e., possessing an anti-scarring effect.

- The combination of the above three effects also explains why amniotic membrane is an ideal substrate to cultivate epithelial progenitor cells of the conjunctiva<sup>21</sup> and the cornea<sup>22</sup> *in vitro*. This effect has been demonstrated in a rabbit model of limbal stem cell deficiency, i.e., the cornea has been rendered deficiency in limbal epithelial stem cells<sup>23</sup> because the life span and clonogenicity of epithelial stem cells have been prolonged.<sup>24</sup> That is why 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.<sup>25</sup> When used in conjunction with conjunctival autografts (to provide some autologous epithelial stem cells), amniotic membrane transplantation is effective to restore the conjunctival surface with symblepharon due to recurrent pterygium.<sup>26</sup> That is also explains why *ex vivo* expanded limbal epithelial progenitor cells by amniotic membrane have been used as a composite graft to restore corneal surfaces inflicted by total and partial limbal stem cell deficiency in human patients.<sup>27</sup>

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- 20 Woo H-M, Kim MS, Kweon O-K, Kim D-Y, Nam T-C, Kim JH. Effects of amniotic membrane on epithelial wound healing and stromal remodelling after excimer laser keratectomy in rabbit cornea. *Br J Ophthalmol*. 2001;85:345-349.
- 21 Cho B-J, Djalilian AR, Obritsch WF, Matteson DM, Chan CC, Holland EJ. Conjunctival epithelial cells cultured on human amniotic membrane fail to transdifferentiate into corneal epithelial-type cells. *Cornea*. 1999;18:216-224.
- Meller D, Tseng SCG. Conjunctival epithelial cell differentiation on amniotic membrane. *Invest Ophthalmol Vis Sci*. 1999;40:878-886.
- 22 Koizumi N, Inatomi T, Quantock AJ, Fullwood NJ, Dota A, Kinoshita S. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. *Cornea*. 2000;19:65-71.
- Koizumi N, Fullwood NJ, Bairaktaris G, Inatomi T, Kinoshita S, Quantock AJ. Cultivation of corneal epithelial cells on intact and denuded human amniotic membrane. *Invest Ophthalmol Vis Sci*. 2000;41:2506-2513.
- 23 Koizumi N, Inatomi T, Quantock AJ, Fullwood NJ, Dota A, Kinoshita S. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. *Cornea*. 2000;19:65-71.
- 24 Meller D, Pires RTF, Tseng SCG. Ex vivo preservation and expansion of human limbal epithelial progenitor cells by amniotic membrane. *Invest.Ophthalmol.Vis.Sci*. 40, S329. 1999. Ref Type: Abstract.
- 25 Tseng SCG, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol*. 1998;116:431-441.
- Anderson DF, Ellies P, Pires RTF, Tseng SCG. Amniotic membrane transplantation for partial limbal stem cell deficiency: Long term outcomes. *Br J Ophthalmol*. 2001;85:567-575.
- 26 Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. *Br J Ophthalmol*. 1998;82:235-240.
- 27 Tsai RJF, Li L-M, Chen J-K. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Eng J Med*. 2000;343:86-93.

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These clinical effects are based on the premises that amniotic membrane facilitates epithelialization, prolongs epithelial stem cell survival, and reduces inflammation and scarring.

### **III. Reported Clinical Uses of Amniotic Membrane for Ocular Surface Reconstruction**

Clinical uses of amniotic membrane for ocular surface reconstruction can be categorized by disease and anatomy, according to the tissue involved.

#### **A. Corneal Surface Disorders without Limbal Stem Cell Deficiency**

##### **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 allogeneic 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 7 studies have been reported. All 7 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 77% (74/96 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<sup>28</sup> first reported that amniotic membrane transplantation results in rapid healing in  $3.9 \pm 2.3$  weeks, complete healing without recurrence in 10/11 consecutive eyes for a follow up of  $9.0 \pm 5.9$  months. One failure case had severe rheumatoid arthritis. Kruse et al<sup>29</sup>

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Schwab IR, Reyes M, Isseroff RR. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea*. 2000;19:421-426.

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

29 Kruse FE, Rohrschneider K, Völcker HE. Multilayer amniotic membrane transplantation for reconstruction

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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<sup>30</sup> reported complete healing in 4/5 eyes. Chen et al<sup>31</sup> reported rapid healing in  $16.6 \pm 9.0$  days in 14/16 eyes of severe neurotrophic ulcers with varying depths for a follow up of  $18.8 \pm 13.0$  months. The four failure cases (presumably due to more severe involvement) required tarsorrhaphy and corneal transplantation to heal the refractory ulceration. Gabric et al<sup>32</sup> reported success of healing corneal ulcers in 8/12 eyes in 1-3 weeks. Hanada et al<sup>33</sup> reported complete healing in  $16.5 \pm 8.0$  days in 8/11 eyes using multiple layers of amniotic membrane to treat deep corneal ulcers with descemetocoele (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<sup>34</sup> 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<sup>35</sup> reported one case report of successful treatment of corneal perforation using amniotic membrane and tissue adhesive.

## 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 potential 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, 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,

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- of deep corneal ulcers. *Ophthalmology*. 1999;106:1504-1511.
- 30 Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. *Br J Ophthalmol*. 1999;83:399-402.
- 31 Chen H-J, Pires RTF, Tseng SCG. Amniotic membrane transplantation for severe neurotrophic corneal ulcers. *Br J Ophthalmol*. 2000;84:826-833.
- 32 Gabric N, Mravacic I, Dekaris I, Karaman Z, Mitrovic S. Human amniotic membrane in the reconstruction of the ocular surface. *Doc Ophthalmol*. 1999;98:273-283.
- 33 Hanada K, Shimazaki J, Shimmura S, Tsubota K. Multilayered amniotic membrane transplantation for severe ulceration of the cornea and sclera. *Am J Ophthalmol*. 2001;131:324-331.
- 34 Letko E, Stechschulte SU, Kenyon KR, Sadeq N, Romero TR, Samson CM, Nguyen QD, Harper SL, Primack JD, Azar DT, Gruterich M, Dohlman CH, Baltatzis S, Foster CS. Amniotic membrane inlay and overlay grafting for corneal epithelial defects and stromal ulcers. *Arch Ophthalmol*. 2001;119:659-663.
- 35 Su CY, Lin CP. Combined use of an amniotic membrane and tissue adhesive in treating corneal perforation: a case report. *Ophthalmic Surg Lasers*. 2000;31:151-154.

Pires et al<sup>36</sup> 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.

### 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<sup>37</sup> 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%).

#### B. Corneal Surface Disorders with 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 review see]. 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.<sup>38</sup> Amniotic membrane was first used

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36 Pires RTF, Tseng SCG, Prabhasawat P, Puangsricharern V, Maskin SL, Kim JC, Tan DTH. Amniotic membrane transplantation for symptomatic bullous keratopathy. *Arch Ophthalmol*. 1999;117:1291-1297.

37 Anderson DF, Prabhasawat P, Alfonso E, Tseng SCG. Amniotic membrane transplantation after the primary surgical management of band keratopathy. *Cornea*. 2001;20:354-361.

38 Tseng SCG. Regulation and clinical implications of corneal epithelial stem cells. *Mol Biol Rep*.

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by Kim and Tseng<sup>39</sup> 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.

A total of 6 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 itself alone without limbal stem cell transplantation is sufficient to restore the corneal surface and improve the vision in a majority of the patients (see below for more details). For unilateral *total* limbal stem cell deficiency, autologous limbal stem cell transplantation can be performed in conjunction with amniotic membrane transplantation.<sup>40</sup> 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 corneal . The overall success is further influenced by the survival of this limbal allograft (see below for more details).

Tseng et al<sup>41</sup> 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<sup>42</sup> 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.

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 the diseases of Stevens-Johnson syndrome in children, Tsubota et al<sup>43</sup> reported successful reconstruction in 3/5 eyes

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1996;23:47-58.

- 39 Kim JC, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea*. 1995;14:473-484.
- 40 Pires RTF, Chokshi A, Tseng SCG. Amniotic membrane transplantation or limbal conjunctival autograft for limbal stem cell deficiency induced by 5- fluorouracil in glaucoma surgeries. *Cornea*. 1999;19:284-287.
- 41 Tseng SCG, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol*. 1998;116:431-441.
- 42 Anderson DF, Ellies P, Pires RTF, Tseng SCG. Amniotic membrane transplantation for partial limbal stem cell deficiency: Long term outcomes. *Br J Ophthalmol*. 2001;85:567-575.
- 43 Tsubota K, Satake Y, Ohyama M, Toda I, Takano Y, Ono M, Shinozaki N, Shimazaki J. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. *Am J Ophthalmol*. 1996;122:38-52.

using amniotic membrane transplantation and keratolimbal allograft. Shimazaki et al<sup>44</sup> 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. Tseng et al confirmed that amniotic membrane transplantation needed to be combined with keratolimbal allograft when there was a total loss of limbal epithelial stem cells.<sup>45</sup> 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<sup>46</sup> 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.<sup>47</sup>

### **C. Conjunctival Surface Reconstruction When a Large Lesion Is Removed During Surgery**

#### **1) Conjunctival Diseases Other Than Pterygium**

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<sup>48</sup> 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 in the following 7 studies for conjunctival surface reconstruction when a large lesion is removed. In general, these 7 studies showed that the defect

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44 Shimazaki J, Yang H-Y, Tsubota K. Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. *Ophthalmology*. 1997;104:2068-2076.

45 Tseng SCG, Prabhawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol*. 1998;116:431-441.

46 Tsubota K, Satake Y, Kaido M, Shinozaki N, Shimmura S, Bissen-Miyajima H, Shimazaki J. Treatment of severe ocular surface disorders with corneal epithelial stem-cell transplantation. *N Eng J Med*. 1999;340:1697-1703.

47 Shimazaki J, Shimmura S, Fujishima H, Tsubota K. Association of preoperative tear function with surgical outcome in severe Stevens-Johnson syndrome. *Ophthalmology*. 2000;107:1518-1523.

48 de Rotth A. Plastic repair of conjunctival defects with fetal membrane. *Arch Ophthalmol*. 1940;23:522-525.

covered by amniotic membrane heals rapidly, and the resultant surface is less inflamed with minimal scarring in those cases with a success. The overall success rate is 89% (77/87 eyes).

Tseng et al<sup>49</sup> 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  $10.9 \pm 9.1$  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.<sup>50</sup> Azuara-Blanco and Dua<sup>51</sup> reported that a successful reconstruction of one case with symblepharon. Meller et al<sup>52</sup> 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). Gabric et al<sup>53</sup> reported success of conjunctival reconstruction in 5/6 eyes with conjunctival scarring. Mejia et al<sup>54</sup> reported successful reconstruction of conjunctival surface in two patients with tumor removal and scar removal. Honavar et al<sup>55</sup> reported successful fornix reconstruction in 9/10 eyes with symblepharon in patients with Stevens-Johnson syndrome in a mean follow up period of  $13.5 \pm 3.8$  months. The complete healing took 1 to 6 weeks. Paridaens et al<sup>56</sup> 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.

## 2) Conjunctival Surface Reconstruction Following Removal of 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

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- 49 Tseng SCG, Prabhasawat P, Lee S-H. Amniotic membrane transplantation for conjunctival surface reconstruction. *Am J Ophthalmol.* 1997;124:765-774.
- 50 Prabhasawat P, Tseng SCG. Impression cytology study of epithelial phenotype of ocular surface reconstructed by preserved human amniotic membrane. *Arch Ophthalmol.* 1997;115:1360-1367.
- 51 Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. *Br J Ophthalmol.* 1999;8339:399-402.
- 52 Meller D, Maskin SL, Pires RTF, Tseng SCG. Amniotic membrane transplantation for symptomatic conjunctivochalasis refractory to medical treatments. *Cornea.* 2000;19:796-803.
- 53 Gabric N, Mravacic I, Dekaris I, Karaman Z, Mitrovic S. Human amniotic membrane in the reconstruction of the ocular surface. *Doc Ophthalmol.* 1999;98:273-283.
- 54 Mejia LF, Acosta C, Santamaria JP. Use of nonpreserved human amniotic membrane for the reconstruction of the ocular surface. *Cornea.* 2000;19:288-291.
- 55 Honavar SG, Bansal AK, Sangwan VS, Rao GN. Amniotic membrane transplantation for ocular surface reconstruction in Stevens-Johnson syndrome. *Ophthalmology.* 2000;107:975-979.
- 56 Paridaens D, Beekhuis H, van Den Bosch W, Remeyer L, Melles G. Amniotic membrane transplantation in the management of conjunctival malignant melanoma and primary acquired melanosis with atypia. *Br J Ophthalmol.* 2001;85:658-661.

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 6 studies used amniotic membrane transplantation for conjunctival surface reconstruction following removal of primary or recurrent pterygium. For primary pterygium, Prabhasawat et al<sup>57</sup> 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<sup>58</sup> 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.<sup>59</sup> Kim et al<sup>60</sup> reported a recurrence rate of 18% in 11 primary pterygium. Ma et al<sup>61</sup> 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.

Recurrent pterygium represents a more aggressive disease. In the study conducted by Prabhasawat et al<sup>62</sup>, the recurrence rate is 37.5% for recurrent pterygium, which is much higher than 9.5% using conjunctival autografts for recurrent pterygium. Gabric et al<sup>63</sup> reported a 30%

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- 57 Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. *Ophthalmology*. 1997;104:974-985.
- 58 Solomon A, Pires RTF, Tseng SCG. Amniotic membrane transplantation after extensive removal of primary and recurrent pterygia. *Ophthalmology*. 2001;108:449-460.
- 59 Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. *Ophthalmology*. 1997;104:974-985.
- 60 Kim JC, Lee D, Shyn KH. Clinical uses of human amniotic membrane for ocular surface diseases. In: Lass JH, ed. *Advances in Corneal Research*. New York: Plenum Press; 1997:117-134.
- 61 Ma DH-K, See L-C, Liau S-B, Tsai RJF. Amniotic membrane graft for primary pterygium: comparison with conjunctival autograft and topical mitomycin C treatment. *Br J Ophthalmol*. 2000;84:973-978.
- 62 Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. *Ophthalmology*. 1997;104:974-985.
- 63 Gabric N, Mravacic I, Dekaris I, Karaman Z, Mitrovic S. Human amniotic membrane in the reconstruction

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recurrence in 10 eyes with recurrent pterygium using amniotic membrane grafts. Subsequently, Solomon et al<sup>64</sup> 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.<sup>65</sup> 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<sup>66</sup> reported that no recurrence in 9 eyes with recurrent pterygium, and Shimazaki et al<sup>67</sup> reported no recurrence in 4 eyes with recurrent pterygium.

### 3) Conjunctival Surface Reconstruction for Glaucoma and Sclera Melt

The anti-inflammatory and anti-scarring effects of amniotic membrane prompted Barton et al<sup>68</sup> to investigate in rabbits the efficacy of maintaining glaucoma filtration procedure. Fujishima et al<sup>69</sup> reported the success of maintaining trabeculectomy using amniotic membrane transplantation in conjunction with 0.4 mg/ml of mitomycin C application. Budenz et al<sup>70</sup> 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. Rodriguez-Ares et al<sup>71</sup> reported a single case of successful reconstruction of conjunctival surface and sclera in a patient with Marfan's syndrome with extensive scleral defect.

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- of the ocular surface. *Doc Ophthalmol.* 1999;98:273-283.
- 64 Solomon A, Pires RTF, Tseng SCG. Amniotic membrane transplantation after extensive removal of primary and recurrent pterygia. *Ophthalmology.* 2001;108:449-460.
- 65 Prabhasawat P, Barton K, Burkett G, Tseng SCG. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. *Ophthalmology.* 1997;104:974-985.
- 66 Kim JC, Lee D, Shyn KH. Clinical uses of human amniotic membrane for ocular surface diseases. In: Lass JH, ed. *Advances in Corneal Research.* New York: Plenum Press; 1997:117-134.
- 67 Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. *Br J Ophthalmol.* 1998;82:235-240.
- 68 Barton K, Budenz D, Khaw PT, Tseng SCG. Glaucoma filtration surgery using amniotic membrane transplantation. *Invest Ophthalmol Vis Sci.* 2001;in press.
- 69 Fujishima H, Shimazaki J, Shinozaki N, Tsubota K. Trabeculectomy with the use of amniotic membrane for uncontrolled glaucoma. *Ophthalmic Surg Lasers.* 1998;29:428-431.
- 70 Budenz DL, Barton K, Tseng SCG. Amniotic membrane transplantation for repair of leaking glaucoma filtering blebs. *Am J Ophthalmol.* 2000;130:580-588.
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#### D. Reconstruction of Both Corneal and Conjunctival Surfaces

One of the devastating and challenging ophthalmic emergencies is chemical burns to both corneal and conjunctival surfaces. Chemical, especially alkali, burns result in severe inflammation, which becomes relentless and extends into a chronic stage. As a result, granulation mixed with necrosis ensues which invariably leads to prominent scarring. Scarring in the corneal surface will reduce the vision, while in the conjunctiva will cause motility restriction and surface breakdown due to constant mechanical microtrauma, exposure, or dryness. Conventional therapies use various medical treatments to suppress inflammation and encourage wound healing with a limited success. The majority of patients with chemical burns, especially those with a grade worse than 1, end up with severe ocular surface failure in the later stage. Little or no surgical means has been practiced for such patients at the acute stage. Sorsby et al<sup>72</sup> in 1946 and 1947, respectively, were the first using chemically processed amniotic membrane to treat chemical burns at the acute stage. Probably due to the nature of extensive chemical processing, the resultant dry membrane graft needed to be applied repeatedly to achieve a successful result. They reported that the earlier the surgical intervention, the better the visual outcome, and the shorter the hospitalization. Using the said method to prepare and preserve amniotic membrane, Kim et al<sup>73</sup> reported a favorable outcome in treating acute alkali burns in rabbits. Their results concur with the generally observed effects of amniotic membrane, i.e., in reducing inflammation and scarring, and in promoting the epithelial wound healing.

Meller et al<sup>74</sup> first reported the clinical efficacy in using amniotic membrane transplantation to treat acute chemical or thermal burns in human patients whose burns were graded as 2 to 3 (7 eyes) and 4 (6 eyes). For a follow-up of  $8.8 \pm 4.7$  months, 11/13 eyes (85%) showed epithelialization within 2 to 5 weeks ( $23.7 \pm 9.8$  days), and final visual acuity improved for more than 6 lines (n=6), 4 to 5 lines (n=2), and 1 to 3 lines (n=2). There was only one eye experiencing symblepharon formation. That is, the cicatricial (scarring) complication is markedly reduced. Eyes with burns of grade 2 to 3 showed more visual improvement than those with burns with grade 4. Eyes with grade 2 to 3 burns did not develop limbal stem cell deficiency, supporting the notion that amniotic membrane transplantation can maintain and help expand the remaining limbal epithelial stem cells in these eyes. All eyes with grade 4 burns developed partial or total limbal stem cell deficiency eventually, indicating that amniotic membrane transplantation helps reduced cicatricial complications but cannot prevent limbal stem cell deficiency. These eyes will require stem cell transplantation in the future. The fact that cicatricial complication is reduced will increase the outcome of future corneal surface reconstruction when

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72 Sorsby A, Symons HM. Amniotic membrane grafts in caustic burns of the eye. *Br J Ophthalmol.* 1946;30:337-345.

Sorsby A, Haythorne J, Reed H. Further experience with amniotic membrane grafts in caustic burns of the eye. *Br J Ophthalmol.* 1947;31:409-418.

73 Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits protease activity on wound healing following acute corneal alkali burns. *Exp Eye Res.* 1998;70:329-337.

74 Meller D, Pires RTF, Mack RJS, Figueiredo F, Heiligenhaus A, Park WC, Prabhasawat P, John T, McLeod SD, Steuhl KP, Tseng SCG. Amniotic membrane transplantation for acute chemical or thermal burns. *Ophthalmology.* 2000;107:980-990.

combined with limbal stem cell transplantation. Sridhar et al<sup>75</sup> also reported successful reconstruction in two cases, i.e., one with chemical and the other with thermal burns at the acute stage using amniotic membrane transplantation.

#### **D. Complications**

There have not been any report showing microbial infections directly linked with amniotic membrane transplantation. Kim et al<sup>76</sup> 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<sup>77</sup> 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.

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75 Sridhar MS, Bansal AK, Sangwan VS, Rao GN. Amniotic membrane transplantation in acute chemical and thermal injury. *Am J Ophthalmol.* 2000;130:134-137.

76 Kim JC, Lee D, Shyn KH. Clinical uses of human amniotic membrane for ocular surface diseases. In: Lass JH, ed. *Advances in Corneal Research.* New York: Plenum Press; 1997:117-134.

77 Gabler B, Lohmann CP. Hypopyon after repeated transplantation of human amniotic membrane onto the corneal surface. *Ophthalmology.* 2000;107:1344-1346.