

Topics in OCULAR ANTIINFLAMMATORIES

A CONTINUING
MEDICAL EDUCATION
PUBLICATION



ISSUE 23

The Role of Inflammation in Diabetic Eye Disease

DANIEL F. KIERNAN, MD Some degree of inflammation is involved in nearly all forms of diabetic eye disease. Prompt recognition that antiinflammatory therapy may be warranted, particularly in cases of diabetic macular edema, may lead to improved outcomes.

Vascular endothelial growth factor (VEGF) inhibitors are a significant therapeutic advancement in the treatment of diabetic retinopathy (DR) and diabetic macular edema (DME).¹ But it is clear from multiple lines of evidence that DR and DME are complex, multifactorial conditions for which anti-VEGF injections are not a panacea.² Indeed, anti-VEGF therapy is ineffective or insufficiently effective for a substantial portion of patients.³⁻⁵ Given the pathophysiological role of inflammation in DR and (especially chronic) DME, antiinflammatory drugs can be an essential component of treatment.

INFLAMMATORY MEDIATORS

In patients with diabetes, elevated blood glucose and advanced glycosylation end products cause dysregulation of the retinal vasculature, including pericyte and endothelial cell loss, capillary dropout, and consequent ischemia. This dysregulation, in turn, leads to the release of a host of proinflammatory growth factors, including VEGF, and cytokines.²

Aqueous and vitreous samples taken from patients with DR and DME consistently show elevated levels of inflammatory molecules such as interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, interferon- γ , intercellular adhesion molecule (ICAM)-1, and monocyte chemoattractant protein (MCP-1) versus normal con-



FIGURE 1 Color montage stereo photographs of a patient's right and left fundi demonstrating moderate non-proliferative DR and hard yellow exudates characteristic of clinically significant diabetic maculopathy. (Images courtesy of Dr. Kiernan.)

trols.⁶ Further, the levels of these cytokines, chemokines, and adhesion molecules are found to be increased in proportion with disease severity.⁶

While VEGF may be a dominant contributor to pathologic neovascularization in DR and DME, other factors, including inflammatory mediators, are likely involved. Treatment with anti-VEGF agents, while effective, may not entirely address the underlying inflammatory processes involved in these diseases.

EVIDENCE FROM PIVOTAL STUDIES

In the phase 3 RISE and RIDE trials, patients with DME who were randomized to receive monthly injections of active drug (0.3 or 0.5 mg ranibizumab) showed strong visual acuity

See INSIDE for:
Antiinflammatory Effects of Amniotic Membrane
by Scheffer CG Tseng, MD, PhD

TARGET AUDIENCE This educational activity is intended for ophthalmologists and ophthalmologists in residency or fellowship training.

LEARNING OBJECTIVES Upon completion of this activity, participants will be able to:

1. Summarize the evidence pointing to inflammation as a component of diabetic eye disease.
2. Apply evidence from published studies to the antiinflammatory treatment of diabetic macular edema.
3. Explain amniotic membrane therapy's role in the treatment of inflammatory ocular surface disease, specifically dry eye disease (DED).
4. Describe amniotic membrane's therapeutic actions and ophthalmic uses.

EDITORIAL BOARD / FACULTY ADVISORS

Marguerite B. McDonald, MD, FACS, practices at Ophthalmic Consultants of Long Island, and is a clinical professor of ophthalmology at the New York University School of Medicine. She is also an adjunct clinical professor of ophthalmology at Tulane University Health Sciences Center.

Victor L. Perez, MD, is a professor of ophthalmology at the Duke University School of Medicine. He is also the director of Duke Eye Center's Ocular Immunology Center and Ocular Surface Program.

Topics in Ocular Antiinflammatories is jointly sponsored by Candeo Clinical/Science Communications, LLC, and the University of Florida College of Medicine. This publication is administered by an independent editorial board and supported by an unrestricted educational grant from Shire.

Copyright 2018 Candeo Clinical/Science Communications, LLC. All rights reserved. Neither the University of Florida nor Candeo Clinical/Science Communications, LLC, assumes any responsibility for injury or damage to persons or property arising from the use of information or ideas contained in this publication.

CME REVIEWER
MATTHEW J. GRAY, MD
Assistant Professor
Department of Ophthalmology
University of Florida College of Medicine

UF Continuing Medical Education
UNIVERSITY OF FLORIDA

gains and reductions in central foveal thickness.⁵ Those who had initially been randomized to receive sham injections were able to crossover and receive ranibizumab 0.5 mg after 24 months. Interestingly, at 36 months, anatomical improvements similar to the original treatment groups were seen in the group originally randomized to sham (average OCT thickness of 194.1 μ m in the sham/0.5 mg group, versus 223.4 μ m in the 0.3 mg group and 201.9 μ m in the 0.5 mg group). But the visual acuity gains in the group originally randomized to sham did not catch up to those in the two active treatment groups.³ The proportions of patients who had gained at least 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters from baseline to 36 months were 19.2% for sham/0.5 mg, 36.8% for 0.3 mg, and 40.2% for 0.5 mg in RIDE; and 22.0%, 51.2%, and 41.6%, respectively, in RISE.³

Looking at the effect of a single year of monthly ranibizumab treatment, the mean number of letters gained was 2.8 in the sham/0.5 mg group, versus 10.6 letters in the 0.3 mg group and 11.1 letters in the 0.5 mg group (though the groups at these timepoints were not fully comparable, given the delay in treatment for the original sham group).³ The results of the delayed treatment group portray an overall picture of an ongoing inflammatory condition that results in permanent architectural and/or neurodegenerative changes, including neural cell loss and damage, fibrosis, and pigmentary changes. This points to the benefit of earlier treatment and perhaps to more comprehensive antiinflammatory management.

The Diabetic Retinopathy Clinical Research Network (DRCR.net)'s Protocol I explored the effects of ranibizumab 0.5 mg plus prompt (within 1 week) or deferred (within \geq 24 weeks) focal/grid laser photocoagulation; sham injection plus prompt laser; and intravitreal triamcinolone injection 4 mg plus prompt laser. The overall outcomes favored ranibizumab with either prompt or deferred laser, though interestingly, the outcomes for triamcinolone plus laser were comparable to the ranibizumab groups for pseudophakic patients.^{7,8}

TOPICS IN OCULAR ANTIINFLAMMATORIES, ISSUE 23

STATEMENT OF NEED

The control of ocular inflammation is a critical aspect of medical and surgical ophthalmic practice. Despite their side effects, antiinflammatory drugs are used to treat a very wide range of conditions throughout the eye, from ocular surface disease and allergic conjunctivitis to posterior segment conditions. Use of antiinflammatory agents is also critical in ocular surgery, contributing greatly to patient comfort and positive outcomes.

The ocular antiinflammatory landscape is changing as research reveals more about the role of inflammation in a range of ocular conditions and as new antiinflammatory agents enter the market.^{1,2} Twenty years ago, for example, the idea of using a topical corticosteroid to treat dry eye and/or allergic conjunctivitis was viewed with alarm; today, it is accepted practice.

Although corticosteroids and nonsteroidal antiinflammatory drugs (NSAIDs) have been the mainstays of the ocular anti-inflammatory armamentarium, a number of new agents with novel mechanisms of action (and new ocular drug delivery systems) have come to market or are being made ready for market.^{3,4}

As indications expand and change, and as new drugs, formulations, and delivery systems become available, clinicians require up-to-date protocols for drug selection and use. Such protocols are also needed for routine (but nevertheless off-label) uses of corticosteroids and NSAIDs because important differences in efficacy, safety, and tolerability exist between these classes and among formulations within each of these classes.^{5,6}

By putting the latest published evidence into the context of current clinical practice, *Topics in Ocular Antiinflammatories* equips ophthalmologists to maintain competencies and narrow gaps between their actual and optimal inflammation management practices, across the range of clinical situations in which current and novel ocular antiinflammatories may be used.

REFERENCES

1. Song JS, Hyon JY, Lee D, et al. Current practice pattern for dry eye patients in South Korea: a multicenter study. *Korean Journal of Ophthalmology*. 2014;28(2):115-21.
2. Ciulla TA, Harris A, McIntyre N, Jonescu-Cuypers C. Treatment of diabetic macular edema with sustained-release glucocorticoids: intravitreal triamcinolone acetonide, dexamethasone implant, and fluocinolone acetonide implant. *Expert Opin Pharmacother*. 2014;15(7):953-9.
3. Maya JR, Sadiq MA, Zapata LJ, et al. Emerging therapies for noninfectious uveitis: what may be coming to the clinics. *J Ophthalmol*. 2014;2014:310329.
4. Sheppard JD, Torkildsen GL, Lonsdale JD, et al, and the OPUS-1 Study Group. Lifitegrast ophthalmic solution 5.0% for treatment of dry eye disease: results of the OPUS-1 phase 3 study. *Ophthalmology*. 2014 Feb;121(2):475-83.
5. Fong R, Leitritz M, Siou-Mermet R, Erb T. Loteprednol etabonate gel 0.5% for postoperative pain and inflammation after cataract surgery: results of a multicenter trial. *Clin Ophthalmol*. 2012;6:1113-24.
6. Singer M, Cid MD, Luth J, et al. Incidence of corneal melt in clinical practice: our experience vs a meta-analysis of the literature. *Clin Exp Ophthalmol*. 2012;S1:003.

OFF-LABEL USE STATEMENT

This work may discuss off-label uses of medications.

GENERAL INFORMATION

This CME activity is sponsored by the University of Florida College of Medicine and is supported by an unrestricted educational grant from Shire.

The University of Florida College of Medicine designates this activity for a maximum of 1 *AMA PRA Category 1 Credit™*. There is no fee to participate in this activity. In order to receive CME credit, participants should read the report, and then take the posttest. A score of 80% is required to qualify for CME credit. Estimated time to complete the activity is 60 minutes. On completion, take the test online at <http://cme.ufl.edu/ed/self-study/toai/>. System requirements for this activity are: For PC users: Windows® 2000, XP, 2003 Server, or Vista; Internet

Explorer® 6.0 or newer, or Mozilla® Firefox® 2.0 or newer (JavaScript™ and Java™ enabled). For Mac® users: Mac OS® X 10.4 (Tiger®) or newer; Safari™ 3.0 or newer, Mozilla® Firefox® 2.0 or newer; (JavaScript™ and Java™ enabled). Internet connection required: Cable modem, DSL, or better.

DATE OF ORIGINAL RELEASE September 2018. Approved for a period of 12 months.

ACCREDITATION STATEMENT

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of the University of Florida College of Medicine and Candeo Clinical/Science Communications, LLC. The University of Florida College of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

CREDIT DESIGNATION STATEMENT

The University of Florida College of Medicine designates this enduring material for a maximum of 1 *AMA PRA Category 1 Credit™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

EDITORIAL BOARD/FACULTY ADVISORS

Marguerite B. McDonald, MD, FACS, practices at Ophthalmic Consultants of Long Island, and is a clinical professor of ophthalmology at the New York University School of Medicine. She is also an adjunct clinical professor of ophthalmology at Tulane University Health Sciences Center. Dr. McDonald is a consultant for Allergan, Alcon, Bausch + Lomb, BlephEx, FOCUS Laboratories, Shire, and J&J Vision.

Victor L. Perez, MD, is a professor of ophthalmology at the Duke University School of Medicine. He is also the director of Duke Eye Center's Ocular Immunology Center and Ocular Surface Program. Dr. Perez is a consultant for Allergan, Shire, EyeGate, and TopiVert. He is also a stock shareholder for EyeGate.

Matthew J. Gray, MD, is an assistant professor in the department of ophthalmology at the University of Florida College of Medicine. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

Daniel F. Kiernan, MD, practices at Ophthalmic Consultants of Long Island in Lynbrook, Mineola, and Rockville Centre, NY. He has received grant/research support from Allergan, Alimera Sciences, and Graybug. Dr. Kiernan is also a consultant for Allergan, Alimera Sciences, Genentech, Regeneron, and Mallinckrodt Pharmaceuticals, and is on the speakers' bureau for Allergan, Alimera Sciences, Genentech, and Regeneron.

Scheffer CG Tseng, MD, PhD, is the medical director of the Ocular Surface Center in Miami, FL and serves as the chief technology officer of TissueTech, Inc. in Miami, FL. He states that he has received grant/research support from National Institutes of Health and the National Eye Institute. Dr. Tseng is also a stock shareholder for TissueTech, Inc.

DISCLAIMER

Participants have an implied responsibility to use the newly acquired information to enhance patient outcomes and professional development. The information presented in this activity is not meant to serve as a guideline for patient care. Procedures, medications, and other courses of diagnosis and treatment discussed or suggested in this activity should not be used by clinicians without evaluation of their patients' conditions and possible contraindications or dangers in use, applicable manufacturer's product information, and comparison with recommendations of other authorities.

COMMERCIAL SUPPORTERS

This activity is supported by an unrestricted educational grant from Shire.

A post-hoc analysis of pooled data from the two ranibizumab treatment arms found a significant association between the improvement in best-corrected visual acuity (BCVA) at 12 weeks and at 1 and 3 years.⁴ Researchers stratified patients by level of treatment response (change in BCVA from baseline to week 12): < 5 letter improvement, 5 to 9 letters of improvement, or ≥ 10 letters of improvement. They found that only a minority of those with the lowest level of improvement at 12 weeks would go on to experience clinically significant gains in BCVA with continued treatment over the ensuing 1 to 3 years.⁴ Thus, where initial response to anti-VEGF therapy is minimal, looking to alternative or adjunctive treatments, including corticosteroids, makes sense.

IMAGING AND MONITORING

At present, spectral-domain optical coherence tomography (SD-OCT) is the gold-standard for assessing treatment response in DME; fluorescein angiography is also commonly used to thoroughly characterize the extent of nonperfusion and vascular leakage. Ultra-widefield photography and angiography are increasingly favored to help identify areas of peripheral ischemia, which may contribute to more center-involved disease by generating cytokines and other inflammatory mediators (Figures 1 and 2).⁹ Some researchers have found that DME with subfoveal neuroretinal detachments detectable on SD-OCT may represent a specific disease presentation associated with a higher concentration of inflammatory mediators, such as IL-6, in the vitreous.⁶ Hyperreflective spots or foci may be another SD-OCT indicator of DR and DME severity.⁶

A number of studies have looked at a retinal “ischemic index” (essentially, a ratio of the nonperfused retinal area to the total retinal area), demonstrating that eyes with larger areas of retinal nonperfusion and neovascularization tended to have more severe, recalcitrant DME.⁹ Ultra-widefield imaging may be helpful in guiding targeted sectoral laser therapy to address areas of peripheral ischemia which may indirectly contribute to more central disease and might otherwise be missed. DRCR.net researchers are conducting a prospective study to explore the impact of ultra-widefield imaging on the ability to predict disease progression over time.¹⁰

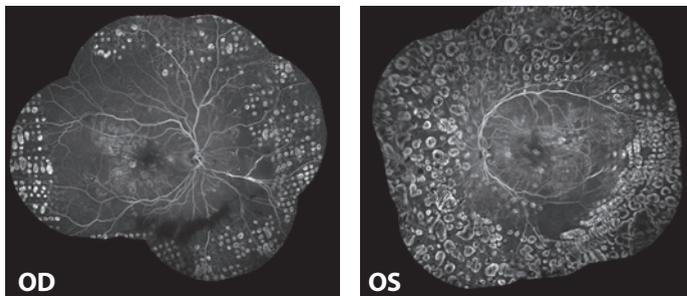


FIGURE 2 Widefield montage fluorescein angiographic images of a patient’s right and left fundi demonstrating ischemic peripheral areas due to proliferative DR treated with pan-retinal photocoagulation. Shadowing due to an inferior vitreous hemorrhage is present OD. (Images courtesy of Dr. Kiernan.)

CORE CONCEPTS

- ◆ Inflammation is among the pathophysiological mechanisms at work in diabetic eye disease.
- ◆ Elevated levels of inflammatory mediators are found in aqueous and vitreous samples taken from patients with DR and DME and increase in proportion to disease severity.
- ◆ Evidence from clinical studies of anti-VEGF treatments suggest that damage due to inflammatory changes can result from delayed treatment.
- ◆ In general, initial response to anti-VEGF therapy in DME is predictive of long-term response.
- ◆ A switch to or addition of corticosteroid may be warranted in cases of poor initial response to anti-VEGF therapy.
- ◆ There is limited evidence that adding steroid to anti-VEGF in persistent DME is of benefit to visual acuity.
- ◆ SD-OCT and ultra-widefield angiography are allowing for more detailed, comprehensive examination and treatment monitoring.
- ◆ Intravitreal corticosteroid implants offer long-term antiinflammatory coverage but are accompanied by risks of cataract progression and IOP elevation.
- ◆ Novel anti-VEGF agents and combinations, plus alternative routes of drug administration, may be on the horizon for DR and DME treatment.

ANTIINFLAMMATORY TREATMENT OPTIONS

Currently, in the absence of DME, the only approved treatment for diabetic retinopathy is ranibizumab 0.3 mg. However, it is clear from clinical studies of the other available anti-VEGF agents and the corticosteroids approved for DME that these treatments provide secondary improvements in diabetic retinopathy severity scores.^{5,11,12}

Most of my patients with center-involving, clinically significant DME receive anti-VEGF injections as first-line therapy, and I turn to corticosteroids when there is an inadequate anatomical and/or visual response after the first two to three monthly injections. Options include preservative-free triamcinolone, used off-label; the biodegradable dexamethasone intravitreal implant (Ozurdex 0.7 mg, Allergan), which lasts 3 to 6 months; and the fluocinolone acetonide (FAC) intravitreal implant (Iluvien 0.19 mg, Alimera Sciences), which lasts up to 36 months.

Because of the risk of elevated intraocular pressure (IOP) resulting in incisional surgery during the FAME clinical trial, the FDA indication for the FAC implant specifies a prior trial of corticosteroids to test for a clinically significant rise in IOP.¹³⁻¹⁵ In my practice, if IOP is not significantly elevated after 3 to 4 weeks with topical corticosteroids, intravitreal triamcinolone, or the dexamethasone implant, or is controllable with IOP-lowering drops, I may proceed to the longer-acting FAC implant for patients who have persistent fluid as seen on optical

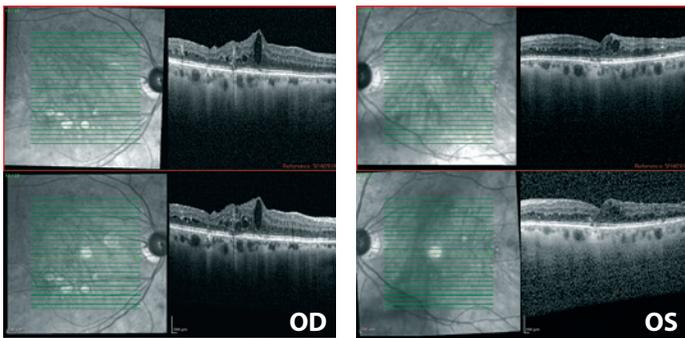


FIGURE 3 Spectral domain optical coherence tomography images of a patient's right and left maculae demonstrating persistent DME before (top images) and after intravitreal anti-VEGF injections. The decision was made to initiate intravitreal corticosteroid therapy to manage this persistent DME. (Images courtesy of Dr. Kiernan.)

coherence tomography and therefore may need longer-term antiinflammatory treatment (Figure 3).

GENERAL CONSIDERATIONS

Similar to the results from clinical trials, I find that about 30% of the patients I treat with the dexamethasone intravitreal implant develop elevated IOP, which is manageable with topical therapy in the majority of cases. In some situations (eg, when IOP is above 30 mm Hg while on ocular hypotensive drops) I may refer patients to a glaucoma specialist for consideration of laser trabeculoplasty or other options.

In addition to monitoring IOP, cataract progression is a concern for patients receiving intravitreal corticosteroid. However, clinical trials of these implants generally show good visual results for patients who start out phakic and undergo cataract surgery.^{14,15}

When cataracts are visually significant and surgery is indicated, I prefer to have a longer-acting antiinflammatory product on board, such as the dexamethasone or the long-acting FAc implant. This helps to ensure that the retina is as flat as possible and may also help limit the inflammatory impact of the surgery. Another consideration for cataract surgery, which typically prompts a referral, is preoperative dry eye disease.¹⁶ Dry eye symptoms are common in patients with diabetes, and there is evidence of altered tear parameters and reduced corneal nerve density in these patients, both of which can impact surgical outcomes.¹⁶

LOOKING AHEAD

Opportunities remain to address the underlying inflammatory component of DR and DME with sufficient power as well as appropriate safety. The manifestations of diabetes and diabetic eye disease vary from patient to patient, and we do not currently have a way to readily measure, for example, the level of inflammatory cytokines in affected eyes. So, with current technology, it is difficult to customize a proactive antiinflammatory treatment plan for DR or DME; our approach is necessarily to let response to initial treatment guide us.

One systemic therapy that has been available for decades,

subcutaneous adrenocorticotrophic hormone (ACTH) gel, has a broad indication for ophthalmic inflammatory conditions, and I have used it successfully in some patients with recalcitrant posterior uveitis or multifocal choroiditis who happen to be diabetic. ACTH binds to melanocortin receptors and is thought to exert its antiinflammatory effects primarily by stimulating glucocorticoids and potentially through other mechanisms.^{17,18} Its use is uncommon in ophthalmology largely because there is a paucity of relevant, controlled clinical data about it.^{17,18} In general, adverse events are likely to be similar in nature to those of systemic corticosteroids.¹⁷

On the horizon a micro-injection preparation of triamcinolone acetonide, intended for administration to the suprachoroidal space, is being investigated in phase 2 trials as an adjunct to aflibercept for DME.¹⁹ Further, a novel anti-VEGF agent, brolicizumab, which has shown robust phase 3 data for neovascular age-related macular degeneration, will also be investigated in the treatment of DME.^{20,21} Other agents that combine VEGF and angiopoietin-2 inhibition are also being investigated clinically for DME.^{22,23}

REMAINING QUESTIONS

Ultimately, the data to guide our adjunctive use of antiinflammatory agents to treat DR and DME is somewhat limited. The recently published DRCR.net Protocol U explored the addition of a dexamethasone implant to ongoing ranibizumab injections in patients with persistent DME after at least three anti-VEGF injections.^{24,25} Patients were randomized to receive ranibizumab plus dexamethasone implant or ranibizumab plus sham injection. This investigation found that at 24 weeks, although the addition of dexamethasone had a more pronounced effect on retinal thickness, it did not improve visual acuity over and above continued ranibizumab therapy.^{24,25} Likewise, a recent systematic review found low-quality evidence (mostly from trials involving triamcinolone and bevacizumab) to suggest that, in general, the combination of anti-VEGF and corticosteroid injections did not provide significant visual benefit over monotherapy.²⁶

Yet inflammation management in DR and DME, particularly recalcitrant cases, remains important. While adjunctive therapy may or may not be warranted in many cases, a switch to corticosteroid treatment because of inadequate response to anti-VEGF therapy or, occasionally, systemic safety concerns, is often the reasonable choice.

Daniel F. Kiernan, MD, practices at Ophthalmic Consultants of Long Island in Lynbrook, Mineola, and Rockville Centre, NY. He has received grant/research support from Allergan, Alimera Sciences, and Graybug. Dr. Kiernan is also a consultant for Allergan, Alimera Sciences, Genentech, Regeneron, and Mallinckrodt Pharmaceuticals, and is on the speakers' bureau for Allergan, Alimera Sciences, Genentech, and Regeneron. Medical writer Jennifer Zweibel of Candeo Clinical/Science Communications, LLC, assisted in the preparation of this manuscript.

KIERNAN REFERENCES continue on page 9

Antiinflammatory Effects of Amniotic Membrane

SCHEFFER CG TSENG, MD, PHD Over the past two decades, amniotic membrane has proved to be an effective treatment modality for an increasing number of ocular surface diseases with inflammation. Its ophthalmic applications should continue to grow, thanks to the new discovery about a crucial active component and, accordingly, a greater understanding of the tissue's antiinflammatory and other therapeutic actions.

Amniotic membrane, the innermost layer of the fetal membrane complex, consists of a thick basement membrane, a thin epithelium, and an avascular stroma. It is a versatile biological tissue with clinical uses in multiple medical fields including ophthalmology. The ophthalmic indications for amniotic membrane therapy encompass a wide range of conjunctival and corneal conditions where inflammation control and tissue repair are desirable (Table I). A few examples are persistent epithelial defects, corneal ulcers, pterygium, chemical burns, recurrent epithelial erosion, Stevens-Johnson syndrome, and limbal stem cell deficiency. This article focuses on amniotic membrane's antiinflammatory effect and its therapeutic implications in the management of ocular surface disorders.

HISTORICAL PERSPECTIVES

Amniotic membrane use is not a recent development in ophthalmology. Its history goes back to 1940, when De Rötth

TABLE I

Ophthalmic Indications for Amniotic Membrane Therapy

AS A SURGICAL GRAFT

- Pterygium and pinguecula
- Bulbar conjunctival scarring
- Conjunctivochalasis
- Infectious keratitis and scleritis
- Symptomatic bullous keratopathy
- Partial limbal stem cell deficiency
- Total limbal stem cell deficiency with limbal transplantation
- Glaucoma surgery (filtering bleb leakage, tube exposure, etc.)

AS A BIOLOGICAL BANDAGE/PATCH

- Refractory ulcerative keratitis
- Acute chemical/thermal burns
- Stevens-Johnson syndrome
- Persistent epithelial defect
- Recurrent corneal erosion
- Dry eye disease
- Haze after refractive surgery

CORE CONCEPTS

- ◆ Amniotic membrane therapy is widely used in the management of ocular surface disorders with inflammation. It has two distinct types of ocular surface usage: as a permanent surgical graft or as a biological bandage or patch.
- ◆ In ocular surface repair or reconstruction, the most important properties of human amniotic membrane include the antiinflammatory effect through suppression of proinflammatory cells and the ability to promote epithelialization. Amniotic membrane also possesses potent antiscarring and antiangiogenic actions that are beneficial for surface restoration in a variety of ocular surface pathologies.
- ◆ The mechanism of action of amniotic membrane's therapeutic effects involves certain signaling molecules and regulatory factors present in the tissue. A recently identified matrix component named HC-HA/PTX3 is likely one of the active components responsible for amniotic membrane's antiinflammatory and other therapeutic actions.
- ◆ The HC-HA/PTX3 complex exerts broad antiinflammatory actions and regenerative healing by orchestrating multiple cellular actions on the ocular surface.
- ◆ Cryopreservation devitalizes living cells, retains the native architecture of amniotic membrane and maintains the quantity and activity of key biological signals present in the tissue such as HC-HA/PTX3A. Dehydration, by comparison, is a harsher process with detrimental effects on the tissue's properties.
- ◆ Amniotic membrane therapy has shown promise for a role in the management of DED. Its clinical benefits for moderate-to-severe DED include not only reduction of ocular surface inflammation but also regeneration of corneal nerves.

first documented the successful use of a fetal membrane graft for repair of conjunctival defects.¹ Several years later, Sorsby and colleagues reported using amniotic membrane as a temporary patch to treat ocular burns.^{2,3} These initial studies, however, did not lead to further investigation into amniotic membrane's ocular use. For five decades, the method was largely neglected and hardly ever mentioned in the literature.

In 1995, my colleague Kim and I reported using amniotic membrane transplantation for ocular surface reconstruction in a rabbit limbal stem cell deficiency model.⁴ We found that glycerin-preserved human amniotic membrane promotes corneal recovery in rabbits after total corneal epithelial removal and a limbal lamellar keratectomy. It appeared that the amniotic membrane graft acted as a substrate to support regeneration of limbal stem cells, just as good topsoil in the garden supports the growth of newly planted seeds. This discovery helped explain why limbal stem cell transplantation performed back then would sometimes fail with no obvious

reason for failure, a problem that we had struggled for years to address. The rabbit study suggested for the first time that the answer lies in the lack of a supporting stromal environment, and that amniotic membrane transplantation as an adjunctive measure can improve the outcome of stem cell transplantation. This is now a well-established concept.

In retrospect, our work marked the revival of amniotic membrane transplantation in the field of ophthalmology. From then on, the technique truly took hold and, with the advent of the cryopreservation method, in 1997, grew rapidly in popularity. Numerous clinical studies took place, establishing the evidence basis for the therapy's efficacy.

THE MOLECULAR MECHANISM

The therapeutic effect of amniotic membrane is believed to originate from its innate wound healing, antiscarring, antiangiogenic, and, most importantly, its antiinflammatory properties. Indeed, when cryopreserved amniotic membrane received approval through Request for Designation from the Food and Drug Administration (FDA) for ocular surface reconstruction, in 2001, it was approved to promote healing through antiinflammatory, antiscarring, and antiangiogenic effects.

Amniotic membrane has long been thought to produce these biological actions through the release of certain regulatory factors within the tissue.⁵ However, it is only recently that the molecular identity of such crucial components has begun to come to light. One may naturally assume that amniotic membrane's complex actions is likely to be based on a symphony of biological molecules, but our cumulative research over more than a decade suggests otherwise. In 2006, we first reported that amniotic membrane stromal matrix promotes apoptosis of activated macrophages to suppress proinflammatory responses.^{6,7} We then demonstrated in an experimental study in 2008 that such antiinflammatory activity is retained in soluble amniotic membrane extract.⁸ In 2009, we successfully identified and purified heavy chain-hyaluronic acid/pentraxin 3 (HC-HA/PTX3) for the first time from amniotic membrane extract. This HC-HA/PTX3 complex is a unique matrix component that retains fresh amniotic membrane's multifactorial antiinflammatory actions.⁹

Further studies have shown that HC-HA/PTX3 is a key signaling molecule in amniotic membrane that orchestrates multiple cellular actions to suppress inflammation and promote regenerative healing.⁷ The complex promotes apoptosis of macrophages and suppresses lipopolysaccharide-induced macrophage infiltration of the cornea. It facilitates apoptosis of activated neutrophils and lymphocytes and inhibits activation of helper T cells. Such antiinflammatory effect in turn leads to enhanced wound repair and decreased scar formation, and the HC-HA/PTX3 complex confers a direct antiscarring effect as well—by inhibiting activities of fibroblasts on the ocular surface.⁹⁻¹² The molecule also has profound influences on the behavior of vascular endothelial cells and, as a result, an antiangiogenic effect.

MODES OF USAGE

On the ocular surface, amniotic membrane can be used

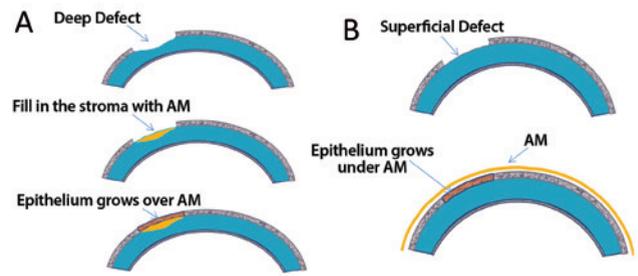


FIGURE 1 Amniotic membrane's modes of action. A) As a permanent graft, to fill in stromal defect and allow the epithelium to grow on top of the graft. B) As a biological bandage, to control ocular surface inflammation and promote healing. (Modified from Hosam Sheha, MD, PhD & Scheffer CG Tseng, MD, PhD, *Clinical Atlas Clinical of Procedures in Ophthalmic and Oculofacial Surgery* 2012.)

as either a permanent surgical graft or a temporary biological bandage or patch (Figure 1). When used as a permanent graft, amniotic membrane is surgically transplanted—by either sutures or fibrin glue—to the cornea, conjunctiva, or other tissue planes to fill in a void for repair or reconstruction (Figure 2A and B). The membrane will be remodeled during wound healing to become part of the host tissue, a process that may take a whole month. When applied as a temporary ocular surface dressing, the membrane exerts its biological actions without full integration into the host tissue (Figure 2C). It needs to be removed upon healing, though in cases where inflammation is intense, the tissue tends to undergo dissolution.

Amniotic membrane's structural integrity and biological properties are affected differently by the processing methods. In the US, preservation of amniotic membrane relies on two main types of techniques: cryopreservation, which involves freezing of the tissue at very low temperatures; and dehydration by heat drying following high salt extraction. This dehydrated amniotic membrane has not been cleared by FDA to possess the aforementioned anti-inflammatory and antiscarring action, and is cleared only as a wound covering. In a recent study of a head-to-head analysis of cryopreservation and dehydration using biochemical and functional assays, we found that cryopreserved amniotic membrane maintains the quantity and activity of HC-HA/PTX3, while dehydrated tissues are

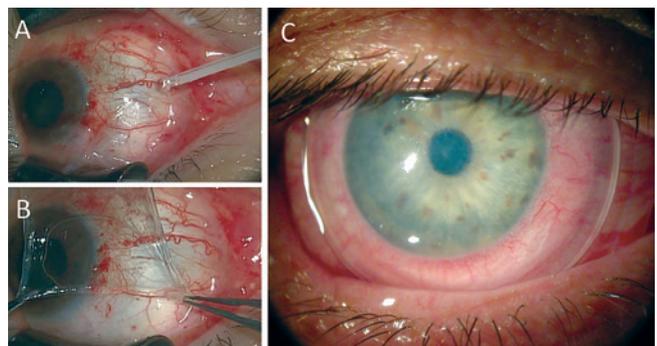


FIGURE 2 Sutureless application of amniotic membrane. A and B) Amniotic membrane graft secured with fibrin glue after pterygium excision. C) Self-retained biological bandage applied to the ocular surface for neurotrophic keratitis. (Images Courtesy of Hosam Sheha, MD, PhD.)

structurally compromised and almost completely deficient in the complex's integral components.¹³

A UNIQUE ANTIINFLAMMATORY THERAPY

The HC-HA/PTX3 complex stands out as a unique class of antiinflammatory therapy. Conventional antiinflammatory agents, such as corticosteroids, nonsteroidal antiinflammatory drugs (NSAIDs), and cyclosporine, tend to target a specific action of one particular cell type. The HC-HA/PTX3 complex in the amniotic membrane, by comparison, acts on neutrophils, macrophages, and lymphocytes that mediate both innate and adaptive immune responses. By downregulating multiple types of inflammatory cells and addressing various cellular actions, HC-HA/PTX3 exerts a broader and likely more potent antiinflammatory effect. Meanwhile, HC-HA/PTX3 has not been associated with any adverse effects, perhaps because it affects activated cells but spares normal, resting cells.¹⁴

The broad antiinflammatory action of HC-HA/PTX3 means that amniotic membrane therapy could be an effective treatment for different types of ocular surface inflammation. Of course, inflammation arises from the immune system's responses to an injury or a noxious insult, and that underlying injury or insult will need to be addressed separately. When treating an infection, for example, one must consider using antimicrobials besides quelling the inflammatory response set off by the infection. This is precisely where amniotic membrane therapy can be particularly useful in treating ocular surface disorders—as a complementary adjunctive therapy for inflammation control and enhanced healing.

A THERAPEUTIC ROLE FOR DRY EYE

In recent years, researchers have begun to explore the use of amniotic membrane therapy for the treatment of dry eye disease (DED), in which inflammation plays a central pathogenic role. The common, multifactorial disease has many different anatomical forms, but all enter the final common vicious circle of inflammation leading to ocular surface disruption and clinical signs and symptoms. Various antiinflammatory therapies are used to treat DED, including corticosteroids, cyclosporine, and, most recently, lifitegrast. Still, there is a refractory population that does not respond well to conventional forms of treatment and needs new therapeutic regimens.

Recent studies provide increasing evidence supporting the use of amniotic membrane as a therapy for moderate-to-severe DED.¹⁵⁻¹⁷ In a retrospective chart review at multiple clinical sites, amniotic membrane treatment rapidly reduced signs and symptoms of DED and accelerated corneal healing in patients with refractory DED.¹⁷ The favorable outcomes are attributed to amniotic membrane's potent inflammatory effect, but the treatment's benefits for DED patients likely go beyond that.

In the course of chronic DED, inflammation of the ocular surface can cause corneal nerve endings to gradually degenerate and, eventually, disappear.¹⁸ Corneal nerves, by mediating blink and tearing reflexes, play an important role in maintaining the healthy state of the corneal epithelium and a stable tear film. Injury or loss of these sensory nerves will further ag-

gravate tear film insufficiency and the self-perpetuating cycle of ocular surface deterioration. Rich in neurotrophic factors, particularly nerve growth factor (NGF), amniotic membrane is thought to facilitate regeneration of corneal nerves.¹⁹ This is supported by the efficacy amniotic membrane therapy has demonstrated in not only restoring corneal sensitivity in eyes with DED but also pain control in patients with neuropathic corneal pain.^{15,20}

Amniotic membrane's regenerative effect distinguishes it from conventional antiinflammatory therapies such as cyclosporine, corticosteroids, and NSAIDs, which are potentially deleterious to corneal nerves.²¹⁻²³ Another advantage of amniotic membrane is its lasting effectiveness. While conventional therapies require a daily maintenance dose to be effective, amniotic membrane is able to produce more than 3 months of symptom improvement after a single placement of several days.¹⁵⁻¹⁷ Such sustained therapeutic effect is likely resulted from regeneration of corneal nerves and raises the possibility of reducing the use of concomitant topical medications to minimize the risk of side effects.

A PROMISING FUTURE

Today, cryopreserved amniotic membrane comes in many different forms to reduce inflammation and improve ocular surface health, including a sutureless, in-office therapeutic device that is available for use in primary eye care. The field is continuing to evolve with biologic graft therapies and even pharmaceutical products. One may envision that the platform technology based on HC-HA/PTX3 may help develop new therapeutics in reducing inflammation to orchestrate regenerative healing.

Scheffer CG Tseng, MD, PhD, is the medical director of the Ocular Surface Center in Miami, FL and serves as the chief technology officer of TissueTech, Inc. in Miami, FL. He states that he has received grant/research support from National Institutes of Health and the National Eye Institute. Dr. Tseng is also a stock shareholder for TissueTech, Inc. Medical writer Ying Guo, MBBS, PhD, assisted in the preparation of this manuscript.

REFERENCES

1. De Rötth A. Plastic repair of conjunctival defects with fetal membranes. *Arch Ophthalmol*. 1940. 23:522-55.
2. Sorsby A, Symons HM. Amniotic membrane grafts in caustic burns of the eye. *Br J Ophthalmol*. 1946(30):337-45.
3. Sorsby A, Haythorne J, Reed H. Further experience with amniotic membrane grafts in caustic burns of the eye. *Br J Ophthalmol*. 1947(31):409-18.
4. Kim JC, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea*. 1995;14:473-84.
5. Tseng SC, Espana EM, Kawakita T, et al. How does amniotic membrane work? *Ocul Surf*. 2004;2(3):177-87.
6. Li W, He H, Kawakita T, Espana EM, Tseng SC. Amniotic membrane induces apoptosis of interferon-gamma activated macrophages in vitro. *Exp Eye Res*. 2006;82(2):282-92.
7. Tseng SC. HC-HA/PTX3 purified from amniotic membrane as novel regenerative matrix: insight into relationship between inflammation and regeneration. *Invest Ophthalmol Vis Sci*. 2016;57(5):ORSFh1-8.

TSENG REFERENCES continue on page 9

This CME activity is sponsored by the University of Florida College of Medicine and is supported by an unrestricted educational grant from Shire. Participants must score at least 80% on this exam in order to receive credit. The University of Florida College of Medicine designates this enduring material for a maximum of 1 *AMA PRA Category 1 Credit™*. To take this exam and obtain credit, please take the test online at <http://cme.ufl.edu/ed/self-study/toai/>. Expires: August 31, 2019.

1. Which of the following inflammatory mediators have been found at elevated levels in aqueous or vitreous samples from diabetic eyes?
 - A. IL-6
 - B. IFN- γ
 - C. ICAM-1
 - D. All of the above
2. The fluocinolone acetonide intravitreal implant:
 - A. Delivers a bolus of drug that tapers over 12 months
 - B. Delivers a continuous dose of FAc over 36 months
 - C. Is a common first-line treatment for center-involving DME
 - D. Is a common first-line treatment for proliferative DR
3. What is the retinal ischemia index?
 - A. The number of clock-hours affected by nonperfusion
 - B. The ratio of perfused to nonperfused retina
 - C. The area of nonperfused retina over the total retinal area
 - D. The percentage of the retinal periphery that is nonperfused
4. Which of the following statements is correct about HC-HA/PTX3?
 - A. It retains the antiinflammatory actions of amniotic membrane
 - B. It induces apoptosis of both activated and resting inflammatory cells
 - C. It regulates innate but not adaptive immune responses
 - D. Its synthetic form is under investigation in clinical trials
5. In addition to inflammation reduction, what therapeutic effect does amniotic membrane exert in the treatment of DED?
 - A. Increased tear production
 - B. Improved corneal sensitivity
 - C. Accelerated epithelial healing
 - D. Both B and C
6. Amniotic membrane has been found to suppress the proinflammatory responses of:
 - A. Macrophages
 - B. Fibroblasts
 - C. Vascular endothelial cells
 - D. Epithelial cells
7. Which of the following SD-OCT features may be associated with DME severity?
 - A. Subfoveal neuroretinal detachments
 - B. Epiretinal membrane
 - C. Hyperreflective spots
 - D. Both A and C
8. Which of the following is an advantage of amniotic membrane compared with conventional topical antiinflammatory therapies?
 - A. Broad antiinflammatory actions
 - B. Long duration of action
 - C. Few detrimental effects on the ocular surface
 - D. All of the above
9. Post-hoc analysis of the DRCR.net Protocol I data found which of the following?
 - A. Response to the first ranibizumab injection predicted outcomes at 5 years
 - B. Response after the first three ranibizumab injections predicted outcomes at 3 years
 - C. There was no clear association between early and long-term response to ranibizumab
 - D. Ranibizumab was superior to sham injection at 3 months
10. Which of the following is NOT an initial FDA-approved claim for cryopreserved amniotic membrane in ocular surface reconstruction?
 - A. Antiinflammatory effect
 - B. Antiscarring effect
 - C. Antiangiogenic effect
 - D. Analgesic effect

KIERNAN REFERENCES from page 4

1. Virgili G, Parravano M, Evans JR, Gordon I, Lucenteforte E. Anti-vascular endothelial growth factor for diabetic macular oedema: a network meta-analysis. *Cochrane Database Syst Rev.* 2017 Jun 22;6:CD007419.
2. Semeraro F, Cancarini A, dell’Omo R, Rezzola S, Romano MR, Costagliola C. Diabetic retinopathy: vascular and inflammatory disease. *J Diabetes Res.* 2015;2015:582060. doi: 10.1155/2015/582060. Epub 2015 Jun 7.
3. Brown DM, Nguyen QD, Marcus DM, et al. Long-term outcomes of ranibizumab therapy for diabetic macular edema: the 36-month results from two phase III trials: RISE and RIDE. *Ophthalmology.* 2013;120(10):2013-22.
4. Gonzalez VH, Campbell J, Holekamp NM, et al. Early and long-term responses to anti-vascular endothelial growth factor therapy in diabetic macular edema: analysis of Protocol I data. *Am J Ophthalmol.* 2016;172:72-9.
5. Brown DM, Schmidt-Erfurth U, Do DV, et al. Intravitreal aflibercept for diabetic macular edema 100-week results from the VISTA and VIVID studies. *Ophthalmology.* 2015;122(10):2044-52.
6. Vujosevic S, Simo R. Local and systemic inflammatory biomarkers of diabetic retinopathy: an integrative approach. *Invest Ophthalmol Vis Sci.* 2017;58:BIO68-75.
7. Elman MJ, Aiello LP, Beck RW, et al. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology.* 2010;117(6):1064-77.
8. Bressler SB, Glassman A, Almkhatar T, et al. Five-year outcomes of ranibizumab with prompt or deferred laser versus laser or triamcinolone plus deferred ranibizumab for diabetic macular edema. *Am J Ophthalmol.* 2016;164:57-68.
9. Patel RD, Messner LV, Teitelbaum B, Michel KA, Hariprasad SM. Characterization of ischemic index using ultra-widefield fluorescein angiography in patients with focal and diffuse recalcitrant diabetic macular edema. *Am J Ophthalmol.* 2013;155(6):1038-44.
10. DRCR.net. Peripheral diabetic retinopathy (DR) lesions on ultra-widefield fundus images and risk of DR worsening over time. <http://drcrnet.jaeb.org/Studies.aspx?RecID=239>. Accessed May 31, 2018.
11. Wykoff CC, Chakravarthy U, Campochiaro PA, Bailey C, Green K, Cunha-Vaz J. Long-term effects of intravitreal 0.19 mg fluocinolone acetonide implant on progression and regression of diabetic retinopathy. *Ophthalmology.* 2017;124:4:440-9.
12. Iglicki M, Zur D, Busch C, Okada M, Loewenstein A. Progression of diabetic retinopathy severity after treatment with dexamethasone implant: a 24-month cohort study. *Acta Diabetologica.* 2018;55:626-35.
13. Regillo CD, Callanan DG, Do DV, et al. Use of corticosteroids in the treatment of patients with diabetic macular edema who have a suboptimal response to anti-VEGF: recommendations of an expert panel. *Ophthalmic Surg Lasers Imaging Retina.* 2017;48:291-301.
14. Boyer DS, Yoon YH, Belfort R Jr., et al. Three-year, randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with diabetic macular edema. *Ophthalmology.* 2014;121(10):1904-14.
15. Campochiaro PA, Brown DM, Pearson A, et al. Sustained delivery fluocinolone acetonide vitreous inserts provide benefit for at least 3 years in patients with diabetic macular edema. *Ophthalmology.* 2012;119(10):2125-32.
16. Markoulli M, Flanagan J, Tummanapalli SS, Wu J, Wilcox M. The impact of diabetes on corneal nerve morphology and ocular surface integrity. *Ocul Surf.* 2018;16:45-57.
17. Agarwal A, Hassan M, Sepah YJ, Do DV, Nguyen QD. Subcutaneous repository corticotropin gel for non-infectious panuveitis: Reappraisal of an old pharmacologic agent. *Am J Ophthalmol Case Reports.* 2016;4:78-82.
18. Wright AD, Sharma P, Zweifel B, Oh L. Suppression of acute uveitis following treatment with repository corticotropin injection. *J Immunol.* 2017;198(1) (Suppl):127.9.
19. Clearside Biomedical. Suprachoroidal CLS-TA with intravitreal aflibercept versus aflibercept alone in subject with diabetic macular edema (TYBEE). ClinicalTrials.gov Identifier: NCT03126786. <https://clinicaltrials.gov/ct2/show/NCT03126786>. Accessed May 30, 2018.
20. Dugel P, et al. A Comparison of the anatomical efficacy of brolucizumab and aflibercept in neovascular age-related macular degeneration (nAMD): an analysis over 16 weeks of matched treatment in the HAWK and HARRIER studies. Presented at the Association for Research in Vision and Ophthalmology Annual Meeting, Honolulu, HI; April 29-May 3, 2018.
21. Novartis Pharmaceuticals. Study of Efficacy and Safety of Brolucizumab vs. Aflibercept in Patients with Visual Impairment Due to Diabetic Macular Edema (KESTREL). ClinicalTrials.gov Identifier: NCT03481634. <https://clinicaltrials.gov/ct2/show/NCT03481634>. Accessed May 30, 2018.
22. Regeneron Pharmaceuticals. Anti-vascular Endothelial Growth Factor pLUs Anti-angiopoietin 2 in Fixed combination therapy: Evaluation for the Treatment of Diabetic Macular Edema (RUBY). ClinicalTrials.gov Identifier: NCT02712008. <https://clinicaltrials.gov/ct2/show/NCT02712008>. Accessed May 30, 2018.
23. Hoffman-La Roche. A Study of RO6867461 in Participants with Center-involving Diabetic Macular Edema (BOULEVARD). ClinicalTrials.gov Identifier: NCT02699450. <https://clinicaltrials.gov/ct2/show/NCT02699450>. Accessed May 30, 2018.
24. Maturi RK, Glassman AR, Liu D, et al. Effect of adding dexamethasone to continued ranibizumab treatment in patients with persistent diabetic macular edema: A DRCR Network phase 2 randomized clinical trial. *JAMA Ophthalmol.* 2018;136(1):29-38.
25. Krick TW, Bressler NM. Recent clinically relevant highlights from the Diabetic Retinopathy Clinical Research Network. *Curr Opin Ophthalmol.* 2018 May;29(3):199-205.
26. Mehta H, Hennings C, Gillies MC, Nguyen V, Campain A, Fraser-Bell S. Anti-vascular endothelial growth factor combined with intravitreal steroids for diabetic macular oedema. *Cochrane Database Syst Rev.* 2018 Apr 18;4:CD011599. doi:10.1002/14651858.CD011599.pub2.

TSENG REFERENCES continued from page 7

8. He H, Li W, Chen SY, Zhang S, et al. Suppression of activation and induction of apoptosis in RAW264.7 cells by amniotic membrane extract. *Invest Ophthalmol Vis Sci.* 2008;49(10):4468-75.
9. He H, Li W, Tseng DY, et al. Biochemical characterization and function of complexes formed by hyaluronan and the heavy chains of inter-alpha-inhibitor (HC*HA) purified from extracts of human amniotic membrane. *J Biol Chem.* 2009;284(30):20136-46.
10. Li W, He H, Chen YT, Hayashida Y, Tseng SC. Reversal of myofibroblasts by amniotic membrane stromal extract. *J Cell Physiol.* 2008;215(3):657-64.
11. Lee SB, Li DQ, Tan DT, Meller DC, Tseng SC. Suppression of TGF-beta signaling in both normal conjunctival fibroblasts and pterygial body fibroblasts by amniotic membrane. *Curr Eye Res.* 2000;20(4):325-34.
12. Tseng, SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. *J Cell Physiol.* 1999;179(3):325-35.
3. Cooke M, Tan EK, Mandrycky C, He H, O’Connell J, Tseng SC. Comparison of cryopreserved amniotic membrane and umbilical cord tissue with dehydrated amniotic membrane/chorion tissue. *J Wound Care.* 2014;23(10):465-74,476.
14. He H, Zhang S, Tighe S, Son J, Tseng SC. Immobilized Heavy Chain-Hyaluronic Acid Polarizes Lipopolysaccharide-activated Macrophages toward M2 Phenotype. *J Biol Chem.* 2013;288(36):25792-803.
15. John T, Tighe S, Sheha H, et al. Corneal nerve regeneration after self-retained cryopreserved amniotic membrane in dry eye disease. *J Ophthalmol.* 2017;2017:6404918.
16. Cheng AM, Zhao D, Chen R, et al. Accelerated restoration of ocular surface health in dry eye disease by self-retained cryopreserved amniotic membrane. *Ocul Surf.* 2016;14(1):56-63.
17. McDonald MB, Sheha H, Tighe S, et al. Treatment outcomes in the DRy Eye Amniotic Membrane (DREAM) study. *Clin Ophthalmol.* 2018;12:677-81.
18. Labbé A, Liang Q, Wang Z, et al. Corneal nerve structure and function in patients with non-sjogren dry eye: clinical correlations. *Invest Ophthalmol Vis Sci.* 2013;54(8):5144-50.
19. Touhami A, Grueterich M, Tseng SC. The role of NGF signaling in human limbal epithelium expanded by amniotic membrane culture. *Invest Ophthalmol Vis Sci.* 2002;43(4):987-94.
20. Morkin ML, Hamrah P. Efficacy of self-retained cryopreserved amniotic membrane for treatment of neuropathic corneal pain. *Ocul Surf.* 2018;16(1):132-8.
21. Namavari A, Chaudhary S, Chang JH, et al. Cyclosporine immunomodulation retards regeneration of surgically transected corneal nerves. *Invest Ophthalmol Vis Sci.* 2012;53:732-40.
22. Lee HK, Ryu IH, Seo KY, Hong S, Kim HC, Kim EK. Topical 0.1% prednisolone lowers nerve growth factor expression in keratoconjunctivitis sicca patients. *Ophthalmology.* 2006;113(2):198-205.
23. Gaynes BI, Onyekwulije A. Topical ophthalmic NSAIDs: a discussion with focus on nepafenac ophthalmic suspension. *Clin Ophthalmol.* 2008;2(2):355-68.