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Focused Review

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Modulation of Fibroblasts in Conjunctival Wound Healing

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Modulating conjunctival wound healing has the potential to improve outcomes after glaucoma filtration surgery and for several ocular disorders, including ocular cicatrial pemphigoid, vernal keratoconjunctivitis, and pterygium. Although anti-inflammatories and antimetabolites have been used with success, these nonspecific agents are not without their complications. The search for novel and more targeted means to control conjunctival fibrosis without such limitations has brought much attention to the regulation of fibroblast proliferation, differentiation, extracellular matrix production, and apoptosis. This review provides an update on where we stand with current antifibrotic agents and outlines the strategies that novel agents use, as they evolve from the bench to the bedside. *Ophthalmology 2017;■:1–14 © 2017 by the American Academy of Ophthalmology*

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Conjunctival Wound Healing in Ocular Disorders

The posterior segment of the eye responds to injury in a similar manner to the central nervous system, referred to as "gliosis." However, the wound-healing response by the conjunctiva, as well as the anterior segment of the eye in general, is comparable to noncentral nervous system tissues and characterized by fibrosis.[1] This response is thought to play a key role in the pathobiology of several ocular conditions. Intrinsic or migratory ocular fibroblasts play a significant role in the wound-healing process. Modulation of fibroblast activity in conjunctival wound healing has the potential to improve the outcome of several ophthalmic conditions. Please refer to the online supplementary material (Table S1, available at www.aaojournal.org) for modulating pharmacological agents not discussed in this review.

Glaucoma Filtration Surgery

The clinical scenario in which wound healing has been most thoroughly investigated and thought to have a profound impact is in glaucoma filtration surgery (GFS). Glaucoma is the second leading cause of blindness worldwide, estimated to have affected 50.6 million people in 2010, with this number increasing to 79.6 million people in 2020.[2] Glaucoma filtration surgery typically is performed for patients with glaucoma refractory to maximal medical therapy. Success of the surgery depends on incomplete scarring of the surgical wound, allowing aqueous to drain from the anterior chamber of the eye to the subconjunctival space. This results in the formation of a filtration bleb, and fluid is slowly absorbed by vessels in the conjunctiva. Failure of glaucoma surgery commonly results from excessive wound healing causing complete scarring at the surgical site. This results in the obstruction of any such drainage and a failure to control intraocular pressure.

Other Conditions in Which Conjunctival Fibrosis Is Important

Among the many other ocular conditions in which conjunctival scarring is important, 3 important conditions come to mind. Ocular cicatrial pemphigoid (OCP), a potentially blinding autoimmune condition, is a subset of the pemphigoid conditions characterized by hyperproliferative conjunctival fibroblasts,[3] larger amounts of collagen,[4] and scarring of the conjunctiva.[5] Second, active vernal keratoconjunctivitis (VKC) is a childhood, self-limiting atopic scarring disorder typified by allergic inflammation

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of the conjunctiva and collagen hyperproduction.[6] Third, pterygium is a conjunctival disorder characterized by fibrotic subconjunctival connective tissue and hypertrophy of overlying conjunctival epithelium.[7] Although its pathogenesis is multifactorial, growth is thought to be mostly secondary to high exposure to ultraviolet irradiation. In vitro studies of fibroblasts from patients with pterygium have shown that they are hyperactive.[8]

Mechanisms of Conjunctival Wound Healing

The pathobiology of wound healing has been reviewed elsewhere and is summarized in Figure 1.[9,10] In essence, wound healing is a series of overlapping phases that follow an insult or injury to the conjunctiva (e.g., surgery). This injury often results in disruption to the vasculature and subsequent leakage of cells (e.g., platelets), proteins (e.g., fibrin), and hormones. The first process is hemostasis, whereby a fibrin clot and platelet plug are formed to maintain the integrity of the vasculature. Activated platelets release a multitude of growth factors, such as platelet-derived growth factor, vascular endothelial growth factor (VEGF), and powerful cytokines such as transforming growth factor β (TGF-β) and interleukins. Particular cytokines have been implicated in ocular pemphigoid[11–13] and VKC.[14–18]

Growth factors and cytokines promote the secondary part of wound healing, the inflammatory phase, marked by the influx of neutrophils, monocytes, and other inflammatory cells. In some ocular conditions, specific inflammatory cells and their chemical mediators are critical to promoting fibrosis (e.g., eosinophils release histamine in VKC). In vitro studies showed that histamine acts on subconjunctival fibroblasts from patients with VKC to increase proliferation, migration, collagen production,[19] and the release of several proinflammatory cytokines.[20] Phagocytic cells, such as neutrophils and monocytes, secrete proteolytic enzymes and promote the debridement of tissues. Much like activated platelets, activated phagocytes elaborate growth factors such as fibroblast growth factor and cytokines such as TGF-β, which are essential for the recruitment, activation, and maintenance of fibroblasts.

The proliferative phase follows and allows for the formation of a granulation tissue beneath the epithelium. It is characterized by hypercellularity, predominantly fibroblasts, and increased fibroblast activity. The 2 key processes of the proliferative phase are angiogenesis, the formation of new blood vessels, and fibrogenesis, the synthesis of loose connective tissue. Growth factors and cytokines are essential to these processes. Vascular endothelial growth factor promotes the formation of new blood vessels, and platelet-derived growth factor is a potent stimulator of ocular fibroblasts.[21,22] Platelet-derived growth factor stimulates inflammatory cells and fibroblasts themselves to release TGF-β, which in turn acts in an autocrine manner on fibroblasts to stimulate their proliferation, migration, and collagen production. Under TGF-β stimulation, fibroblasts also differentiate into myofibroblasts, a contractile phenotype characterized by intracellular α–smooth muscle actin. They enhance expression of extracellular matrix (ECM) proteins and facilitate wound contracture and closure. Cytokines such as interleukin 4 and interleukin 13 have been shown to increase the production of collagen by conjunctival fibroblasts.[23]

The final remodeling phase involves the maturation of the fibrovascular tissue into a mature scar. It is characterized by the activity of matrix metalloproteinases (MMPs) synthesized by fibroblasts, macrophages, and neutrophils. The MMPs mediate selective ECM degradation. Collagen type I replaces collagen type III and becomes cross-linked and dehydrated to culminate in the transformation of cellular granulation tissue into a dense hypocellular scar. The reduction in myofibroblast numbers, via apoptosis, is critical to this phase of wound healing. Prolongation of their survival is a key factor that leads to excessive scarring.

The degree of scarring as opposed to regeneration of the original tissue architecture is determined by the severity of the initial insult and the host's wound-healing response. Modulating fibroblast activity, particularly in the proliferative phase, remains an important mechanism by which clinicians may control the outcome of conjunctival wound healing. Multiple points of regulation exist where fibroblast activity can be modulated: migration, proliferation, transdifferentiation into myofibroblasts, production and secretion of collagen, and apoptosis. This review focuses on the many pharmacologic agents that have been used to attenuate fibroblast activity and conjunctival wound healing.

Pharmacologic Modulation of Conjunctival Fibrosis

Anti-inflammatories

Steroids. Although steroids predominantly modulate wound healing by affecting the inflammatory phase, they also have effects on fibroblasts in the proliferative phase. In vitro studies have shown the multifaceted approach that steroids seem to use to modulate fibroblast activity. Steroids that reduce fibroblast proliferation include triamcinolone (although its effects appear to be short-lived),[24] prednisone,[25] and dexamethasone.[26–29] Dexamethasone has been shown to reduce ocular fibroblast attachment,[27] reduce production of chemokines such as eotaxin-1,[30] and delay wound closure in a wound-healing model.[26]

Much of the data from clinical studies are in the setting of GFS studies, with some of these studies being performed prior to the availability of antimetabolites. An early pilot study highlighted how subconjunctival injection of triamcinolone can reduce intraocular pressure and increase bleb formation after surgery for glaucoma,[31] with follow-up histologic studies suggesting this was a result of necrosis of subconjunctival fibroblasts.[32] However, a retrospective analysis[33] and randomized controlled trial[34] failed to show any benefit of triamcinolone injected intraoperatively.

In a prospective study with 10-year follow-up, postoperative prednisolone (topically applied with or without systemic therapy) resulted in lower intraocular pressure, the use of fewer glaucoma medications, and fewer follow-up