• Review Article •

Mechanisms of fibrosis formation following glaucoma filtration surgery

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Abstract

● Glaucoma filtration surgery (GFS) stands as the most effective intervention for reducing intraocular pressure, a critical component in glaucoma management. Despite its pivotal role, the scarring of the filtration bleb remains the primary impediment to successful GFS outcomes. Perioperative utilization of antimitotics, while frontline in combating fibrosis and modulating the wound healing process, carries the risk of vision-threatening complications. Given the complexity of the wound healing cascade and the potential insufficiency of targeting a single molecule, there is an imperative to expand therapeutic modalities through combination therapies. This review offers a comprehensive elucidation of the fibrogenesis post-GFS, a synthesis unprecedented in the available literature, and aims to inform the broadening of therapeutic strategies for GFS.

● **KEYWORDS:** glaucoma filtration surgery; filtration bleb; fibrosis; mechanisms

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INTRODUCTION

Glaucoma constitutes a progressive neurodegenerative condition of the optic nerve, culminating in profound visual field loss and blindness, thus representing a leading cause of irreversible blindness globally[1]. Currently, the only modifiable risk factor and validated therapeutic target for halting the progression of glaucoma is the reduction of intraocular pressure (IOP). This reduction can be achieved through various interventions including topical drops, oral medications, laser surgeries aimed at augmenting aqueous outflow or diminishing aqueous production, with glaucoma filtration surgery (GFS) being the most efficacious modality for IOP reduction.

GFS creates an alternative aqueous humor drainage pathway from the anterior chamber to the subconjunctival and episcleral space, effectively reducing IOP. Trabeculectomy, the gold standard within GFS procedures, establishes a controlled aqueous leak by excising a portion of the trabecular meshwork and creating a tunnel between the anterior chamber and the subconjunctival space, resulting in what is termed a filtration bleb. While this surgery is crucial in managing glaucoma, it is not without risks of complications. Excessive wound healing and scarring within the conjunctiva and Tenon's capsule post-GFS often lead to surgical failure. Unlike many surgical objectives which aim to heal tissue by restoring normal structure, traditional GFS focuses on modulating wound healing to maintain aqueous flow into the subconjunctival or episcleral space, thus preventing surgical failure[2]. Subconjunctival and episcleral fibrosis continues to be a major obstacle to the success of GFS, with fibrosis at the filtration site potentially hindering aqueous outflow and, consequently, causing an increase in IOP. 5-Fluorouracil (5-FU) and mitomycin C (MMC) are extensively utilized to mitigate ocular fibrosis and are considered frontline agents in clinical practice. They have been shown to enhance bleb survival and significantly improve surgical success rates. Nonetheless, the long-term success rates of bleb maintenance with these antimitotic agents are not as promising as expected. Additionally, the nonspecific mechanisms of these drugs may lead to severe vision-threatening side effects. Complications associated with the broad, nonspecific anti-fibrotic properties of these medications include corneal toxicity, endophthalmitis, bleb leaks, hypotony, and infection[3]. Consequently, there is a compelling need to expand the therapeutic avenues for GFS, incorporating combination therapies or agents with more specific physiological actions and reduced cytotoxicity. This review aims to provide a comprehensive exposition of the mechanisms underlying fibrosis formation post-GFS. It delves into the cascade of wound healing, the regulation of growth

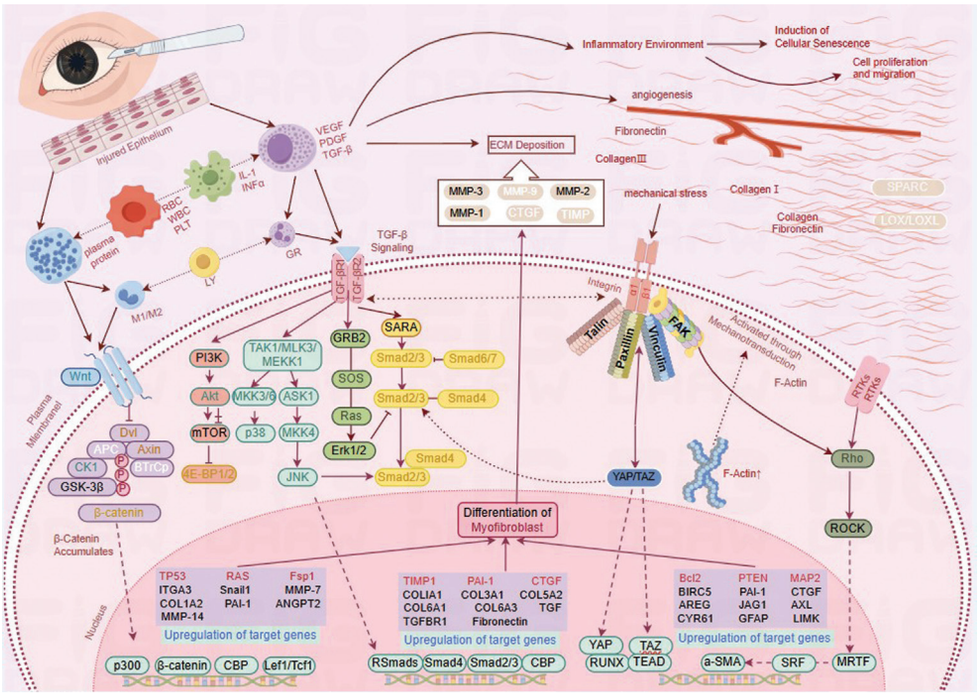

Figure 1 Mechanisms of fibrosis formation following glaucoma filtration surgery (by figdraw) RBC: Red blood cell; WBC: White blood cell; PLT: Platelet; M1/M2: Macrophage 1/macrophage 2; LY: Lymphocyte; GR: Granulocyte; IL-1: Interleukin-1; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; TGF-β: Transforming growth factor-beta; ECM: Extracellular matrix; MMP: Matrix metalloproteinase; LOX: Lysyl oxidase; SPARC: Secreted protein acidic and rich in cysteine; YAP: Yes-associated protein; TAZ: Transcriptional coactivator with PDZ-binding motif; α-SMA: α-Smooth muscle actin; SRF: Serum response factor; MRTF: Myocardin-related transcription factor.

factors and cytokines, the driving forces of intracellular and extracellular signaling pathways, and the biomechanical factors contributing to tissue remodeling. Additionally, it analyzes the merits and pitfalls of current antifibrotic treatment strategies, offering insights to broaden the therapeutic approaches for GFS.

Cascade of Wound Healing The pathophysiological mechanisms activated by tissue fibrosis following injury are consistent across all non-neuronal tissues and organs in the human body. Similar to other surgical interventions, GFS induces tissue trauma. Wound healing is initiated by the activation of the innate immune system, involving a complex and dynamic cascade of events that are distinct yet overlapping. This process encompasses several phases: the coagulative and inflammatory phases, followed by proliferation and repair, and culminating in the remodeling phase[4]. The intricate mechanisms leading to fibrosis formation after GFS are illustrated in Figure 1.

Coagulative and Inflammatory Phases During GFS, the incisions in the conjunctiva and sclera result in connective tissue and vascular damage. This damage triggers the extravasation of plasma proteins (such as fibrinogen, fibronectin, and plasminogen) and blood cells (red cells, white cells, and platelets) from the ruptured vessels. Platelet aggregation then stimulates the intrinsic coagulation cascade, with the activation of clotting factors leading to the conversion of fibrinogen into fibrin, culminating in thrombus formation and hemostasis[5]. Vascular injury during GFS results in the release of clotting substances and a cascade of bioactive molecules such as histamine, serotonin, prostaglandins, leukotrienes, cytokines (like interleukin-1 and interferon-alpha), and growth factors including vascular endothelial growth factor (VEGF), placental growth factor, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF-β)[4]. These substances act as chemotactic agents, directing the migration and attraction of inflammatory cells such as neutrophils, macrophages, and lymphocytes to the site of injury during the inflammatory phase[4]. The infiltrating neutrophils and macrophages, through phagocytosis, play a pivotal role in infection clearance and debris removal from the wound site.

Proliferative and Repair Phases The coagulative and inflammatory phases of wound healing initiate the transition to the proliferative and repair stages, marked by the migration of endothelial cells and fibroblasts to the site of injury. Clinically, this phase is characterized by angiogenesis and the emergence of granulation tissue. There is an increased presence of fibroblasts, while proteolytic enzymes secreted by neutrophils and monocytes facilitate debridement. Activated phagocytes augment the release of growth factors and cytokines; TGF-β activates and sustains fibroblasts, VEGF and bFGF stimulate angiogenesis under hypoxic conditions and

lactic acid formation at the wound site, and PDGF induces the differentiation of fibroblasts into myofibroblasts[6]. Proliferating fibroblasts, influenced by various factors, gradually differentiate into myofibroblasts, which, unlike undifferentiated fibroblasts, mediate wound contraction and form a collagen-rich extracellular matrix (ECM).

Remodeling Phase The final phase of wound healing following GFS involves tissue remodeling and the formation of scar tissue. Over time, vascular regression occurs, and fibroblasts facilitate the cross-linking of type I collagen and elastin, with type I collagen supplanting type III collagen. Within one week to one month, collagen cross-links and dehydrates, increasing tensile strength, leading to fibroblast apoptosis within the granulation tissue, a drastic reduction in cellular components within the ECM, and the eventual transition from a tissue mixed with ECM and vasculature to scar tissue[7-8]. This stage is orchestrated by matrix metalloproteinases (MMPs) synthesized by macrophages, neutrophils, and fibroblasts. A key aspect of this phase is the reduction in the number of myofibroblasts; premature fibroblast apoptosis can result in wound dehiscence, while their prolonged survival may lead to excessive scar formation[7].

Growth Factors and Cytokines Regulation

TGF-β TGF-β is among the most critical growth factors implicated in scarring of the filtration channel post-GFS, and it has been extensively studied due to its multifunctional role in signaling pathways that regulate cellular proliferation, differentiation, adhesion, migration, and apoptosis. TGF-β belongs to a superfamily of polypeptide growth factors and exists as three isoforms in the human body, each exhibiting similar biological activities: TGF-β1, TGF-β2, and TGF-β3. However, their distribution and affinities within the eye differ[9]. Following GFS, the fibroblasts within the conjunctival sac predominantly express TGF-β1 and TGF-β2. Within the eye, TGF-β2 is the most abundant and widely distributed isoform, with its concentration in the aqueous humor significantly surpassing that found in the serum. Conversely, TGF-β1 is chiefly secreted by conjunctival epithelial cells and fibroblasts[9-10]. Within the coagulative and inflammatory stages of the wound healing cascade, tissue damage also activates TGF-β sequestered in the ECM, transforming it into an active form. Upon activation, TGF-β binds to receptors on Tenon's capsule fibroblasts, exerting chemotactic effects on inflammatory cells and intensifying the inflammatory response. Concurrently, TGF-β amplifies the expression of other growth factors, such as connective tissue growth factor (CTGF). In the subsequent proliferative and repair phases, TGF-β stimulates the transformation of fibroblasts into myofibroblasts and boosts the production of type I and type II collagen within the ECM[11]. During the remodeling phase, TGF-β acts to inhibit ECM degradation while simultaneously promoting its deposition, ultimately leading to ECM restructuring and scar formation[12]. TGF-β orchestrates the sequential and interconnected events of wound healing, with its signals being transduced and modulated *via* both the Smad pathway and non-Smad pathways. These signaling pathways form a complex network, exerting mutual influence and dictating the healing outcome.

PDGF PDGF family comprises growth factor isoforms formed by five distinct disulfide-bonded dimers, encoded by four unique polypeptide chains from four different genes. Beyond inducing macrophage and fibroblast proliferation and migration to the site of injury, their upregulation leads to pathological angiogenesis and fibrosis[13]. PDGF works in synergy with TGF-β in an autocrine fashion, stimulating the differentiation of fibroblasts into myofibroblasts[14].

VEGF VEGF serves as a potent mediator of vascular homeostasis, encompassing angiogenesis and endothelial cell permeability, both of which are critical for wound healing. As a key process in wound repair, angiogenesis can be a direct or indirect contributor to fibrosis, with VEGF being a central player. Beyond its role as an effective inducer of angiogenesis, VEGF also promotes the migration of inflammatory cells, such as lymphocytes, macrophages, andneutrophils[4]; it can also bind to VEGF receptors on the surface of Tenon's capsule fibroblasts, enhancing target cell proliferation and migration[15]. Consequently, VEGF, as a growth factor, not only indirectly stimulates fibrosis through angiogenesis but also exerts a direct influence on the activity of fibroblasts[4].

CTGF CTGF, also known as connective tissue growth factor or cellular communication network factor 2, is a member of the immediate early gene family and is ubiquitously distributed across various organs in the human body. Acting as a downstream effector of TGF-β, it is selectively activated by TGF-β and is present within connective tissues, where it is considered a proponent for the progression of fibrosis. Studies have confirmed that CTGF modulates several critical biological functions during the scarring process, including promoting mitosis and fibroblast proliferation, inducing cell adhesion, and enhancing the synthesis of the ECM[16].

Others Additional molecules, including growth factors such as FGF2 and PDGF, cytokines like interleukin-6 , interleukin-8, and tumor necrosis factor-alpha (TNF-α), along with MMPs like MMP-1, MMP-2, and MMP-3, are all implicated in the formation of post-GFS scarring. FGF2, as a regulator of fibroblast activity, promotes the proliferation and differentiation of keratinocytes into fibroblasts and may serve as a therapeutic target to mitigate fibrosis[17]. MMPs, a group of proteolytic enzymes that degrade ECM components, play a central role in collagen contraction and matrix remodeling during wound

healing. These zinc-dependent proteases cleave ECM proteins and degrade molecules that mediate cell-cell and cell-ECM interactions. By proteolytic activation of growth factors and their receptors, MMPs are instrumental in the wound healing process where activated fibroblasts proliferate and migrate to the wound site, remodeling the ECM in an MMP-dependent manner[18]. Research has confirmed that MMPs exhibit their most robust expression between days 3 and 7 post-surgery, corresponding to the early stages of fibroblast formation. During this phase, fibroblasts commence the production of new ECM, while the original ECM components damaged during the filtration surgery are extensively degraded by MMPs. In the later stages of wound healing, as the granulation tissue transitions to fibrosis/scarring, MMPs also play a critical role. This underscores the importance of temporal expression and regulation of MMPs in the dynamic process of ECM remodeling and wound healing following GFS[19].

Cellular and Extracellular Signaling Pathways

Smad pathway Signaling by the TGF-β superfamily is principally mediated through the Smad pathway and non-Smad pathways. Within the Smad signaling cascade, TGF-β, acting as the ligand, binds to its type II receptor and ALK5, forming a receptor complex that activates downstream signaling molecules Smad2 and Smad3. The co-mediator Smad4 is not ligand-restricted and, upon phosphorylation, Smad2 and Smad3 form a trimeric complex with Smad4. This complex then translocates into the nucleus to activate or repress the transcription of target genes regulated by TGF-β[20]. Extensive research indicates that Smad3 is a pivotal mediator within the TGF-β-induced fibrotic signaling pathway, with its overexpression stimulating the basal activity of the promoters for type I collagen α1 and type II collagen α2, thereby playing a role in regulating the expression, synthesis, and contraction of type I collagen[21]. Smad proteins, based on their structure and function, are categorized into at least nine types (Smad1 through Smad9). The receptor-regulated Smads (R-Smads: Smad1, Smad2, Smad3, Smad5, and Smad8) act as downstream effectors activated upon binding to the TGF-β superfamily ligands by serine-threonine kinase receptors[22]; Among these, Smad6 and Smad7 are inhibitory Smads that negatively regulate the signal transduction of the TGF-β family, counteracting the R-Smads[23]. Studies have shown that Smad6 disrupts the non-canonical TGF-β signaling by negatively regulating the TRAF6-TAK1-p38 MAPK/JNK pathway[24].

Rho/ROCK pathway The Ras homolog gene/Rho-associated protein kinase (Rho/ROCK) pathway is integral for regulating myosin, cytoskeletal organization, cell adhesion, and cell motility[25], TGF-β can activate the Rho/ROCK pathway directly, as well as through the Ras system activated by TGF-β. The proliferation, migration, and contraction of various cell types, including endothelial and fibroblast cells, necessitate different healing processes, all of which require the Rho/ROCK pathway to continually rearrange and dynamically regulate the actomyosin cytoskeleton[26]. Rho, a small GTPase and a Ras homolog, regulates the formation of the actin cytoskeleton, while Rho-associated kinases (ROCK I and ROCK II), primary downstream effectors of the Rho family GTP-binding molecules, are serine/threonine protein kinases. Upon Rho activation, ROCK undergoes phosphorylation at multiple amino acid sites, subsequently activating a cascade of signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway[25]. Beyond its role as a cytoskeletal regulator, experimental evidence suggests that ROCKs also play a significant role in gene expression during inflammatory processes. Inhibition of ROCK reduces the activation of NF-κB, which then decreases the production of pro-inflammatory cytokines (such as interleukins and TNF-α) in various inflammatory cells, indicating potential anti-inflammatory effects of ROCK inhibitors[27]. Serum response factor (SRF) and myocardin-related transcription factor (MRTF), downstream in the Rho-ROCK pathway, play a pivotal role in the activation of fibroblasts, while the MRTF-A/SRF transcription pathway is an important upstream regulator of MMPs expression in ocular fibrosis[28].

PI3K/AKT pathway Phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway plays a crucial role in the physiology and pathophysiology of various cell types, regulating not only survival but also migration, proliferation, and apoptosis[29]. The key enzyme PI3K catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate into Phosphatidylinositol 3,4,5-trisphosphate, which facilitates the binding of AKT to 3-phosphoinositide-dependent protein kinase-1, leading to AKT phosphorylation by protein kinase-1[30-31]. AKT, a serine/threonine kinase and a principal intermediary of the PI3K signaling pathway, when activated, triggers a cascade with downstream targets to modulate cellular functions. For instance, AKT regulates cell migration *via* Rac1 and RhoA, enhances cell survival through Bcl-2, and promotes cell proliferation by activating the mammalian target of rapamycin[32]. It has been reported that the PI3K/AKT pathway can be directly activated by TGF-β and plays a significant role in regulating ECM synthesis, inducing the expression of ECM molecules across various cell types[33].

Integrin pathway Integrins are transmembrane receptors found on all nucleated cells, consisting of 18 alpha and 8 beta subunits that assemble into 24 different heterodimeric forms. They are pivotal for mediating signal transduction between the ECM and the intracellular milieu, regulating processes such as cell adhesion, migration, and proliferation[34]. Integrins are

composed of intracellular, transmembrane, and extracellular domains, connecting with cytoskeletal proteins on the inside and binding to specific ligands on the outside through their N-terminal domain to form a ligand-integrin-cytoskeleton transmembrane system. Integrins are categorized by the specificity of their N-terminal domain's binding to ligands, including arginine-glycine-aspartic acid-dependent integrins, laminin-binding integrins, and collagen-binding integrins[35]. As crucial transmembrane receptors and adhesion molecules, integrins convey bidirectional signals that not only mediate cell adhesion but also modulate cell-ECM interactions. In the process of tissue fibrosis, integrin pathways cooperate with various cytokines, such as MMPs, TGF-β, and bFGF, where integrins form a positive feedback loop with TGF-β to mediate collagen remodeling. Some integrins activate TGF-β, converting it from its latent to its active form[36]; conversely, TGF-β can enhance the expression of integrins[37]. Thus, blocking integrin activation or TGF-β can potentially inhibit scarring following GFS.

Other pathways The mitogen-activated protein kinase (MAPK) family, part of the serine/threonine protein kinase family, constitutes a significant portion of the TGF-β-activated non-Smad pathways, encompassing three distinct signaling routes. Extracellular signal-regulated kinases, c-Jun N-terminal kinases, and p38 pathways. Studies have shown that inhibition of the MAPK pathways can significantly reduce scarring of the filtration channel following GFS[38-39]. Wnt/β-catenin signaling pathway, known for regulating inflammatory responses, cell apoptosis, and oxidative stress-mediated tissue fibrosis, has been shown to be activated by TGF-β1, promoting excessive proliferation and activation of Tenon's capsule fibroblasts. Inhibition of the Wnt/β-catenin pathway significantly attenuates the effects of post-GFS TGF-β1 stimulation on the expression of collagen α1 and α-smooth muscle actin (α-SMA), as well as the proliferation of Tenon's capsule fibroblasts[40].

Biomechanical Factors in Tissue Remodeling

Regulation of mechanical stress accumulation Fibroblasts generate contractile forces that are essential in the post-surgical scarring process, with their differentiation into myofibroblasts being a critical event in wound healing. Myofibroblasts deposit ECM proteins and exert contractile forces critical for the remodeling phase of wound healing. A hallmark of myofibroblast differentiation is the expression of α-SMA, characterized by enhanced contractility and abundant ECM production. The synthesis of activated fibroblasts, ECM proteins, growth factors, and integrins are all factors that promote increased α-SMA expression. Increasing evidence suggests that biomechanical factors are major determinants in wound healing and myofibroblast transdifferentiation[41]. In rat models of wound healing, mechanical strain applied to the wound site significantly increases myofibroblast transdifferentiation[42]; *In vitro* studies with fibroblasts embedded in collagen gels have shown that the mechanical stiffness of the gel dictates the TGF-β-induced myofibroblast transdifferentiation and subsequent gel contraction[43]. Cellular mechanotransduction has a profound impact on wound healing and scar formation, processes that can be modulated by the integrin and Rho/ROCK pathways. In the integrin pathway, mechanical stress from the ECM is transmitted to the cell through integrins to the intracellular adhesion complex and actin cytoskeleton, inducing intracellular signals that regulate cell function, promoting adhesion and tissue remodeling[36]. In the Rho/ROCK pathway, the GTPase Rho acts as a molecular switch regulating cellular tension and inducing actin stress fibers and focal adhesions[44]. Rho increases cytoskeletal tension by activating associated ROCKs, thus enhancing myosin light chain phosphorylation and participating in actomyosin contraction[45]. Conversely, ROCK inhibitors decrease cellular tension and prevent the recruitment of α-SMA to stress fibers, leading to the disassembly of actin stress fibers and focal adhesions[46].

Increased stiffness in the microenvironment Myofibroblasts at the site of injury continuously deposit ECM, leading to increased stiffness in the microenvironment and subsequent fibrosis. Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) play central roles in the cellular response to environmental stiffness, a process that also involves the participation of the lysyl oxidase (LOX) family. LOX family are crosslinking substrates of ECM enzymes, such as collagen and elastin, contributing to fibrosis, with members including LOX and LOXL1, LOXL2, LOXL3, and LOXL4, each with distinct biological functions[47]. The stability and localization of YAP and TAZ can be mediated by the Hippo pathway, which constitutes a kinase cascade and is one of the most well-known mechanotransduction pathways. In the absence of mechanical stress, the kinase cascade is active, leading to rapid proteasomal degradation of YAP/TAZ[48]. Conversely, under mechanical stress, the kinase cascade is inactivated, stabilizing YAP/TAZ and promoting their translocation to the nucleus[49]. In the nucleus, YAP/TAZ interact with TEAD transcription factors to drive the transcription of genes associated with cell proliferation, survival, and fibrosis[50]. Research indicates that YAP/TAZ, as primary components of the cellular response to increased microenvironmental stiffness, are closely linked with and regulate TGF-β2 signaling. On one hand, YAP/TAZ are positioned near Smad2/3, with YAP being essential for TGF-β2-mediated phosphorylation and nuclear translocation of Smad2/3. YAP/TAZ directly regulate TGF-β2-mediated fibrosis through modulation of the Smad2/3 pathway. On the

other hand, TGF-β2 stabilizes YAP/TAZ and induces their nuclear translocation, leading to the transcription of fibrotic genes in human primary conjunctival fibroblasts. Inhibitors of YAP/TAZ significantly suppress the fibrotic alterations mediated by TGF-β2 in conjunctival fibroblasts[51].

CONCLUSION

The mechanisms of fibrosis formation following GFS inform a multi-faceted anti-fibrotic strategy encompassing: anti-inflammatory agents, cytokine inhibitors, antiproliferative drugs, intracellular and extracellular signaling pathway inhibitors, ECM modulators, mechanical stress regulators, and novel drug delivery techniques along with biomaterial applications. Anti-inflammatory approaches include broad-spectrum immunosuppressants (such as cyclosporine A and rapamycin), corticosteroids, and nonsteroidal anti-inflammatory drugs. Cytokine inhibitors involve TGF-β inhibitors, anti-PDGF agents, anti-VEGF drugs, and MMP inhibitors. Antimetabolites include MMC, 5-FU, interferons, and gene therapies targeting cell cycle checkpoints. Signaling pathway inhibitors encompass Smad pathway inhibitors, Rho/ROCK inhibitors, PI3K/AKT inhibitors, and MAPK inhibitors. ECM inhibitors comprise LOX family antibodies, fibrinolytic treatments, MRTF/SRF inhibitors, secreted protein acidic and rich in cysteine inhibitors, and YAP/TAZ inhibitors. Mechanical stress regulation techniques involve amniotic membranes, perfluoropropane gas, sodium hyaluronate, and collagen matrix implants. Innovations in drug delivery and biomaterial applications include liposomal delivery systems, nanoparticle carriers, expandable polytetrafluoroethylene implants, and dendrimer implants.

Antimetabolites play a critical role in the anti-fibrotic treatment following GFS, with MMC and 5-FU significantly enhancing the success rate of the surgery. Their effectiveness has been validated in multiple large-scale clinical trials and remains the gold standard in clinical practice[52-53]. However, their use carries an increased risk of postoperative complications. Excessive MMC not only exhibits cytotoxicity but may also upregulate pro-inflammatory, pro-angiogenic, and pro-fibrotic factors. Treatment with 5-FU, compared to MMC, results in fewer side effects, yet the epithelial toxicity from repeated injections cannot be disregarded[53]. Thus, combining MMC and 5-FU with drugs that block their pro-fibrotic targets could offer promising complementary effects and allow for the use of lower doses with fewer side effects.

Cytokine inhibitors targeting TGF-β, VEGF, and PDGF in the treatment of fibrosis post- GFS have shown promising results in improving surgical outcomes, supported by some small comparative and supplementary studies. However, there is still a lack of large randomized clinical trials. Among anti-VEGF drugs, bevacizumab is the only one proven effective in prospective randomized clinical trials, but its use remains controversial, with the optimal route and regimen of administration requiring careful consideration.

Most research on anti-fibrotic treatments post-GFS has focused on molecular changes, utilizing single drugs targeting one molecule to enhance success rates. Yet, given the complexity of wound healing regulation after GFS and the potential for drug resistance with monotherapy, targeting a single molecule may not suffice to halt the fibrotic process. Hence, future approaches will likely necessitate combination drug therapies. Moreover, since biomechanical factors mediate fibrosis, regulating mechanical stress under the conjunctiva and sclera is crucial for postoperative anti-fibrotic success. This could be achieved by implanting physical spacers to prevent fibrosis by separating the conjunctival and sub-scleral tissues, and future research should focus on developing new biomaterials with better biocompatibility. Additionally, traditional anti-fibrotic drug delivery methods and routes result in rapid clearance of drugs from the subconjunctival space. Future studies should prioritize the development of targeted drug delivery systems that minimize foreign body reaction while prolonging the duration of drug activity in the filtration bleb.

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