INTRODUCTION

Bacterial keratitis is a common ocular emergency and is one of the leading causes of blindness worldwide, with *Pseudomonas aeruginosa* being the most frequent causative agent of keratitis. After eradicating the causative bacteria, the corneal damage process continues due to the immunological process of remaining bacterial toxins in the eye. This can result in complications such as corneal fibrosis and neovascularization.

The management goal of keratitis is to eradicate the infective agents, restore the integrity of the ocular surface, and improve corneal clarity. However, the treatment for tissue regeneration following infectious keratitis is still limited. Platelet-rich fibrin (PRF), as a source of growth factors, has emerged as a potential therapeutic option to accelerate wound healing processes.

Bacterial Keratitis

Bacterial keratitis is a corneal epithelium defect with necrosis of the underlying stromal tissue caused by bacterial infection. Bacterial keratitis is a common and significant cause of blindness worldwide, accounting for 4.3% of total blindness cases. Blindness occurs when keratitis cases are not managed properly, resulting in complications such as corneal neovascularization and fibrosis. Bacterial keratitis frequently occurs in developing and developing countries, with an incidence rate of approximately 1.5 to 2 million new cases annually.

The bacteria causing keratitis vary between countries, including *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Coagulase-negative staphylococci,* and *Neisseria meningitidis,* which are largely determined by the local microbial flora as well as geographical location and climates. Among the bacteria causing keratitis, *Pseudomonas aeruginosa* is a highly contagious and difficult-to-manage pathogen.

*Pseudomonas aeruginosa* keratitis often progresses to corneal ulceration. Although prompt antibiotic therapy can kill the bacteria, damage to the cornea persists because the toxin produced by the bacteria triggers an ongoing inflammatory response. Administration of steroid drugs or other anti-inflammatory agents can inhibit this inflammatory response, but no drug can inhibit the activity of the bacterial toxin.

The initial management of bacterial keratitis generally includes the administration of topical antibiotics and mydriatic-cycloplegic agents that aim to reduce pain caused by muscular spasms and prevent iris synechiae. Inadequate antibiotic therapy only weakens the bacteria without permanently killing them, thus more specific antibiotic therapy is required. Broad-spectrum therapy is given as a first step. In cases of bacterial keratitis caused by *P. aeruginosa,* fluoroquinolone antibiotics such as ciprofloxacin, moxifloxacin, and levofloxacin can be used for gram-negative bacteria. However, antibiotic administration must still be tailored to microbiological evaluation such as corneal scrapings, culture, and antibiotic sensitivity.
Corneal Injury Healing Process

Corneal wound healing is a multifaceted process. Typically, the corneal wound healing response can be categorized into four distinct phases, namely homeostasis, inflammation, proliferation, and remodeling. This intricate process involves various growth factors, cytokines, and proteases, which are produced by a diverse array of cells, including epithelial cells, keratocytes in the stroma, inflammatory cells, and lacrimal gland cells.15,16

The homeostatic mechanism initiates promptly after an injury, usually within a few minutes, through platelet activation. This process persists through the rapid formation of fibrin, caused by the activation of the coagulation cascade. The activated platelets stimulate the production of Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), and Transforming Growth Factor Beta-1 (TGF-β1). These factors are crucial in eliminating debris, preventing infection, and inducing cell migration. Furthermore, they are also responsible for promoting cell proliferation, matrix production, and the synthesis of matrix-degrading enzymes.15,17

Following the homeostatic process, an inflammatory phase occurs within 24-48 hours. During this phase, macrophages, T cells, Tumor Necrosing-α (TNF-α), Interleukin (IL)-8, IL-1, and IL-6 can be up-regulated. Neutrophils and monocytes will bind to adhesion molecules on the endothelium, migrate to the wound area using integrins, and then bind to extracellular matrix (ECM) proteins.15,16,17 The subsequent phase is the proliferation phase, which begins at 72-96 hours post-injury. This phase is characterized by increased angiogenesis activity, granulation tissue formation, and an increase in the number of fibroblasts. Fibroblasts will bind collagen and induce Matrix Metalloproteinases (MMPs) migration and production. Finally, the remodeling phase occurs, where wound contraction, deposition, and new matrix synthesis occur. This phase commences after several weeks of treatment and persists for several years.17,18,19

Defects in the corneal stroma can result in damage to the basal membrane. This damage can trigger the release of pro-inflammatory cytokines into the anterior stroma, causing keratocytes to become activated and migrate to the wound area. This process begins 12-24 hours after the wound appears and can be sustained for up to 1 week. During the migration process, keratocytes transform into fibroblasts. Growth factors are then released from the epithelium, and TGF-β causes fibroblasts to transdifferentiate into myofibroblasts. TGF-β1 plays a role in decreasing keratocyte migration but increasing fibroblast chemotaxis and ECM production. Increased ECM production consists of increased stimulation, production, and differentiation of collagen, fibronectin, and proteoglycans, all ECM components. These processes are significant in the field of medicine.16,17,18

The formation of fibroblasts or myofibroblasts through keratocyte transformation will decrease as the wound is filled with fibrotic material. After this, the remodeling phase will begin and be sustained for several weeks to years. The function of the remodeling phase is to restore corneal transparency. However, in pathological conditions, myofibroblasts will release irregular ECM. The formed fibrotic material will contract and cause corneal opacity. The balance between inflammatory mediators, matrix metalloproteinase (MMP), tissue inhibitor of metalloproteinase (TIMP), and the wound area affects the healing outcome of the corneal stroma, which can result in stromal structure regeneration, scar tissue, stromal melting, or neovascularization.17,20

Matrix Metalloproteinases (MMPs) and Tissue Inhibitor of Metalloproteinases (TIMPs)

Matrix metalloproteinases (MMPs) are significant matrix-degrading enzymes produced by corneal epithelium that belong to the family of extracellular endoproteinases that destroy extracellular matrix proteins such as laminin, fibronectin, and collagen.20-26 MMPs are important in normal development, reproduction, and tissue remodeling, as well as in various pathological processes such as cancer, arthritis, angiogenesis, and wound healing, where extracellular matrix destruction is required. MMPs are classified into numerous classes, including gelatinases (MMP-2 and MMP-9), collagenases (MMP-1, MMP-8, and MMP-13), stromelysins (MMP-3, MMP-10, and MMP-11), membrane-type MMPs, and others.20,25,26,27

MMP has the ability to degrade extracellular matrix components and plays a role in wound remodeling. In chronic inflammation, neutrophils and macrophages can secrete MMP and serum proteases at high levels. Regular MMP activity provides benefits for corneal wound healing if produced at the appropriate level, location, and time. However, if MMP activity is too high, it can cause excessive degradation of the extracellular matrix and result in damage to the basal membrane and corneal matrix, leading to tissue damage and ulcer formation, resulting in loss of visual acuity. MMP expression regulation occurs at both the transcriptional level and after transcription by controlling the proenzyme activation and active enzyme inhibition by TIMPs. The severity of clinical damage in ocular surface disorders is closely related to the amount of scar tissue and tissue contraction.25,26,27

MMP-9 exhibits the highest fold change in expression among other genes in keratitis. The high expression of MMP-9 is due to its production by various types of cells, including corneal epithelial cells that aid in wound closure migration, activated keratocytes, macrophage infiltration, and neutrophils. MMP-9 is capable of degrading type IV collagen, and can cleave and activate pro-IL-1β, contributing to corneal inflammation. Active MMP-9 is found in corneal ulcers and ocular surface disorders, indicating that fungal infections in the cornea infiltrate neutrophils into the eye’s anterior chamber. Mitchell et al. described the upregulation of MMP-9 in rats during the early stage of experimental keratomycosis, and MMP-9 expression increased in superficial corneal trauma. Induction of MMP-9 in filamentous fungal keratitis follows the severity of the disease. Corneal injury stimulates an increase in MMP-9 and plays a role in early keratomycosis. Prevention of conidial attachment to the cornea can inhibit inflammation, and administration of metalloproteinase inhibitors is clinically important in preventing corneal ulcers.
and reducing the severity of corneal injury by altering the growth patterns of fungal pathogens and minimizing corneal scarring. 20,25,26,27

Tissue Inhibitor of Metalloproteinase (TIMP) functions as a local inhibitor of enzymes that regulate MMPs, which cause extracellular matrix degradation/damage. TIMPs are divided into four groups: TIMP-1, TIMP-2, TIMP-3, and TIMP-4, which inhibit MMPs by directly attaching to them. TIMP-1 is a protein with a molecular weight of 29 kDa that is produced by keratinocytes, fibroblasts, smooth muscle cells, and endothelial cells. TIMP-2 is a 22-kDa glycoprotein that, like TIMP-1, can block the action of all known MMPs. TIMP-1 is temporally controlled throughout the wound healing process, and an imbalance between TIMP-1 and MMPs has been linked to chronic venous ulcer delayed healing. TIMP-1 levels are sufficiently high in new trauma compared to non-healing wounds, which contain high quantities of activated gelatinases but low TIMP-1 levels. TIMP-1 expression can also be found 3-5 days after acute injury, but not in chronic wounds. Stromal TIMP-1 expression is quite abundant in both acute and chronic wounds, produced by epithelial cells, macrophages, and stromal cells such as fibroblasts. Typically, TIMP-1 protein is detected beneath keratinocytes bordering on non-chronic wounds. 27,28,29

Platelet Rich Fibrin (PRF)

Platelets are non-nucleated cell fragments derived from megakaryocytes and synthesized in the bone marrow by binding cytoplasm regulated by thrombopoietin. Platelet Rich Fibrin (PRF) is an autologous polymerized fibrin matrix that contains a large number of cytokines, growth factors, and other cells. This platelet concentrate does not require anticoagulants or thrombin as they cause the fibrin structure to solidify and act as a mesh to bind platelets and leukocytes during centrifugation. PRF preparation is very simple and requires a 10 mL dry glass tube without anticoagulant and a 24 G needle syringe. Blood is drawn from a vein, 5 ml is placed in a sterile tube without anticoagulant and then centrifuged at 2700-3000 revolutions per minute (rpm) for 10-12 minutes. After centrifugation, the tube is left to stand upright, and several layers can be observed: the bottommost layer, which is red in color, contains red blood cells; the upper layer, which is yellowish-white in color, contains cell-rich plasma; and the middle layer contains a fibrin clot. Following removal from the centrifuge, the upper yellowish-white layer (containing platelet-poor plasma/PPP) is pipetted off. In contrast, the middle layer is retrieved using forceps, approximately 2 mm below the bottommost dividing line. This is what is known as PRF. PRF is mechanically separated from the red blood cell layer in the bottommost layer. The mechanism that occurs here is that fibrinogen initially concentrated in the clear part of the tube, combines with circulating thrombin due to configuration to form fibrin. 30,31,32

Platelet degranulation requires the release of cytokines in order to stimulate cell migration and proliferation in the fibrin matrix, triggering the early stages of wound healing. PRF contains several growth factors such as PDGF, VEGF, and TGF-β, which are slowly released in large quantities over 7 to 28 days during the wound healing process. This is because intrinsic cytokines trapped in the PRF tissue are only released when cicatricial matrix remodeling occurs. 31,32

PRF-produced TGF-β induces fibroblast transdifferentiation into myofibroblasts. Under certain conditions, such as infection, myofibroblasts secrete irregular ECM, leading to corneal fibrosis. The balance between inflammatory mediators, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs), also influences stromal healing outcomes, such as regeneration of stromal structures, scar tissue formation, stromal melting, or neovascularization. The role of PRF derived TGF-β is essential in enhancing fibroblast chemotaxis and ECM production by regulating the expression and activity of matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9) in wound remodeling and healing, where MMPs are regulated or balanced with tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2). 31-40

CONCLUSION

Based on the aforementioned studies, it is demonstrated that PRF has effects on keratitis bacteria as a therapy. PRF was found to slowly release TGF-β in ECM production by regulating MMPs in wound remodeling and healing. An excessive increase of MMP-9 can also result in ECM degradation, so it is regulated by TIMP-1, which appears simultaneously when MMP is active. MMP-9 and TIMP-1 may help wound healing and reduce scar tissue formation, stromal melting, or neovascularization.

ACKNOWLEDGMENT

(-)

CONFLICT OF INTEREST

In this review article, there is no potential conflict of interest.

FUNDING SOURCE

Not applicable.

ETHICAL CLEARANCE

Not applicable.

AUTHOR’S CONTRIBUTION

All authors contributed equally to this review article.

REFERENCES


