REVIEW

Bali Medical Journal (*Bali MedJ)* 2023, Volume 12, Number 3: 2538-2547
P-ISSN.2089-1180, E-ISSN: 2302-2914

The α-smooth muscle actin (α-SMA) and collagen type III expression of Platelet Rich Fibrin (PRF) membrane in conjunctival wound healing: a literature review

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Received: 2023-06-01
Accepted: 2023-07-15
Published: 2023-08-26

ABSTRACT

The platelet-rich fibrin (PRF) membrane represents a new reconstructive material employed in ocular surface procedures. Platelet-rich fibrin (PRF) offers several notable benefits, including its potential to mitigate inflammation, its abundance of growth factors, and its capacity to serve as a transient framework for fibroblast migration. Nevertheless, despite the numerous benefits it offers, PRF is not extensively utilized in comparison to alternative methods like conjunctival autograft. The utilization of conjunctival autograft has been a longstanding approach in ocular surface reconstruction methodologies; however, it is not without its inherent limitations. Therefore, the utilization of the PRF membrane technique presents a viable solution for addressing this particular scenario. This literature review aims to present a comprehensive analysis of the role of PRF in the regulation of inflammation and its potential to facilitate the healing of conjunctival wounds. This review is expected to stimulate further experimental investigations into the influence of α-SMA and collagen type III expression on the efficacy of PRF membranes in promoting conjunctival wound healing.

Keywords: Platelet-rich fibrin membrane, α-SMA, collagen type III, conjunctiva wound healing.
Cite This Article: Wiratama, A.B., Prastyani, R., Wahyuni, I., Valentinus, T., Notobroto, H.B. 2023. The α-smooth muscle actin (α-SMA) and collagen type III expression of Platelet Rich Fibrin (PRF) membrane in conjunctival wound healing: a literature review. *Bali Medical Journal* 12(3): 2538-2547. DOI: 10.15562/bmj.v12i3.4746

INTRODUCTION

The conjunctiva is an ocular structure that contributes to the preservation of the eyeball's surface integrity, the maintenance of corneal clarity, and the safeguarding of the sclera. The evaluation of conjunctival defects can be conducted by considering their dimensions. Simple closures are typically sufficient for small conjunctival defects, whereas larger defects often necessitate the use of an artificial membrane or graft for closure.[1-4]

CONJUNCTIVAL WOUND HEALMING

The process of wound healing in the conjunctiva is characterized by a sequence of phases that occur concurrently following an injury to the conjunctiva, such as a surgical wound, trauma, or disease. The occurrence of a wound or defect in the conjunctiva can be attributed to various factors, including trauma, surgical procedures, or autoimmune disorders. Conjunctival injuries have the potential to induce vascular disturbances, leading to the release of blood cells, including platelets, protein (fibrin), and hormones. This particular injury frequently leads to vascular disorders and manifests as the extravasation of inner vascular components such as thrombocytes (platelets), fibrin proteins, and hormones. The initial stage is known as the haemostasis phase, during which a fibrin clot and platelet plug are generated to preserve the structural integrity of the vascular system. The activated platelet has the ability to release various growth factors, including PDGF, VEGF, and potent cytokines such as transforming growth factor β (TGF-β) and interleukin.[5]

The second stage of conjunctival wound healing, known as the inflammatory phase, is facilitated by the presence of growth factors and cytokines. This phase is characterized by the migration of neutrophils, monocytes, and other inflammatory cells into the wound site. Certain ocular conditions involve the significant involvement of specific inflammatory cells and their chemical mediators, which can lead to the development of fibrosis. For instance, the release of eosinophils is known to contribute to fibrosis in cases of vernal keratoconjunctivitis. The findings of in vitro studies indicate that histamine exerts an influence on subconjunctival fibroblast cells in individuals diagnosed with vernal keratoconjunctivitis, leading

to enhanced proliferation, migration, and collagen synthesis, thereby contributing to the release of certain proinflammatory cytokines. Phagocytic cells, such as neutrophils and monocytes, have the ability to sequester proteolytic enzymes, which can contribute to tissue debridement. The activated phagocytes exhibit similarities to activated platelets in their ability to generate growth factors, such as fibroblast growth factor (FGF), and cytokines, including transforming growth factor-beta (TGF-β). These factors play a critical role in the recruitment, activation, and sustenance of fibroblast cells.[1,2,5]

The proliferative phase denotes the subsequent stage in which granulation tissue is generated beneath the epithelial layer. The condition is distinguished by a heightened presence of cells, particularly fibroblasts, and an augmented level of fibroblast functionality. The proliferative phase encompasses two significant processes, namely angiogenesis, which refers to the development of novel blood vessels, and fibrogenesis, which involves the synthesis of loose connective tissue. Growth factors and cytokines play a crucial role in this biological process. Vascular endothelial growth factor (VEGF) facilitates angiogenesis, the process of generating new blood vessels. On the other hand, platelet-derived growth factor (PDGF) exerts a strong stimulating effect on ocular fibroblasts. Platelet-derived growth factor (PDGF) induces the activation of inflammatory cells and fibroblasts, leading to the secretion of transforming growth factor-beta (TGF-β). Subsequently, TGF-β exerts an autocrine effect on fibroblasts, promoting their proliferation, migration, and synthesis of collagen. Upon stimulation with transforming growth factor beta (TGF-β), fibroblasts undergo differentiation into myofibroblasts, which exhibit a contractile phenotype distinguished by the presence of alpha-smooth muscle actin (α-SMA). They enhance the expression of proteins in the extracellular matrix, thereby facilitating the process of contracture formation and wound healing. According to Zada et al, previous studies have demonstrated that cytokines, specifically interleukin 4 and interleukin 13, have the ability to enhance collagen synthesis in conjunctival fibroblasts.[5]

The final stage of wound healing involves the remodelling phase, wherein the fibrovascular tissue undergoes maturation and transforms into fully developed scar tissue. The observed phenomenon is distinguished by the enzymatic action of Matrix Metalloproteinases (MMPs), which are produced by fibroblasts, macrophages, and neutrophils. Matrix metalloproteinases (MMPs) are responsible for the targeted degradation of specific components within the extracellular matrix (ECM). Collagen type I is responsible for the substitution of collagen type III, as well as the process of crosslinking and dehydration. These processes contribute to the conversion of cellular granulation tissue into a dense hypocellular scar tissue. The induction of apoptosis to decrease the population of myofibroblasts is a critical process during the wound healing phase. The prolongation of myofibroblast viability is a significant determinant that can contribute to the development of excessive fibrotic tissue formation.[2,6]

CONJUNCTIVAL AUTOGRAFT

Conjunctival autograft transplantation is a viable treatment option for individuals with conjunctival defects resulting from trauma, inflammation, or significant conjunctival scarring. The method employed in this procedure involves the utilization of a conjunctival autograft for the purpose of sealing a specific and limited area of damage within the conjunctiva. Large, recurrent pterygium is the primary indication for autograft conjunctival transplantation. Another indication is the presence of a sizable pinguecula, which leads to persistent irritation necessitating the surgical removal and subsequent transplantation of a conjunctival autograft in order to effectively close the significant defect. Complications, namely conjunctival fornix shortening, may arise in various surgical procedures, including retinal detachment surgery, strabismus surgery, and conjunctival tumour or nevus excision surgery. The potential complications arising from the shortening of the conjunctival fornix during this procedure can be effectively managed through the implementation of a conjunctival autograft obtained from a contralateral healthy eye. Regrettably, a number of diseases exhibit bilateral manifestations, including mucous membrane pemphigoid and Stevens-Johnson syndrome. Consequently, the conjunctiva of the contralateral individual's eyes cannot serve as a viable donor due to the presence of damage in both instances. Oral mucosal transplantation, also known as AMT, represents an additional alternative that can be employed in such circumstances.[2]

The conjunctival autograft technique has been found to exhibit a low rate of recurrence. Nevertheless, there are several significant limitations associated with this approach, including the requirement for an extended duration of operation, occurrence of postoperative ocular irritation, and disturbance of the typical anatomy and microenvironment of the conjunctival tissue.[7]

PLATELET RICH FIBRIN (PRF) MEMBRANE

Platelet rich fibrin (PRF) membranes represent the subsequent iteration of platelet concentrates, commonly referred to as PRP. The origins and initial delineation of PRP were established within the domain of haematology. The term "PRP" was introduced by haematologists during the 1970s to refer to blood plasma containing a platelet count higher than that found in peripheral blood. Initially, PRP was utilized as a transfusion product for the treatment of patients suffering from thrombocytopenia. The specimen was acquired from the patient's blood sample prior to undergoing centrifugation.[8] Fibrin possesses homeostatic properties, while PRP exhibits anti-inflammatory characteristics, thereby suggesting its potential to induce cellular proliferation. The utilization of platelet-rich plasma (PRP) has gradually expanded to encompass various medical disciplines, including cardiac surgery, paediatric surgery, gynaecology, urology, plastic surgery, and ophthalmology.[9]

The PRF membrane comprises various components, including cytokines, leukocytes, stem cells, platelets, and tetra-molecular structures. These constituents serve as scaffolds for micro vascularization

and facilitate the transportation of crucial cells involved in tissue regeneration. The present fibrin exhibits enhanced mechanical properties and a reduced rate of remodelling, thus closely resembling the characteristics of blood clots. Platelet-rich fibrin (PRF) exhibits remarkable regenerative capabilities for both soft and hard tissue, while concurrently avoiding any inflammatory responses. The antibacterial and immunological characteristics of platelet-rich fibrin (PRF), which have the potential to induce leukocyte degranulation and stimulate angiogenesis[2,10]

Platelet-rich fibrin (PRF) possesses the capacity to generate a higher aggregate discharge of growth factors compared to platelet-rich plasma (PRP). In addition, it should be noted that the release of platelet-rich fibrin (PRF) is characterized by a slow and extended duration, making it particularly suitable for the purposes of tissue regeneration and stimulation of growth.[11] The process of separating the layers of blood prior to clotting in PRF necessitates a rapid and concise centrifugation procedure. In the platelet-rich layer, a fibrin matrix is generated, which envelops platelets and leukocytes.[2] This matrix, known as platelet-rich fibrin, exhibits a gradual and substantial release of growth factors, such as TGFβ-1, PDGF, and VEGF, over a period of one to two weeks. This phenomenon occurs due to the process of polymerization experienced by platelet-rich plasma (PRP), resulting in the prevention of active platelets from becoming entangled in fibrin networks. Consequently, a significant proportion of growth factors are released within the initial hours. This has been observed in previous studies,[11]

Platelet-rich fibrin (PRF) has been recognized for its role in the wound healing process. It has demonstrated efficacy in promoting the healing of both soft and hard tissues, while also enhancing the body's immune response through the proper organization of the fibrin matrix and direct migration of stem cells. Furthermore, PRF has been found to be a safe and effective treatment for descemetocele. In addition to its therapeutic benefits, PRF grafts also serve as a mechanical framework that facilitates cell proliferation, differentiation, and migration, all of which are crucial for tissue regeneration. The combined mechanical and chemotactic functions of PRF membranes make them suitable as an autologous biomaterial for the reconstruction, repair, and maintenance of ocular surfaces.[12]

The PRF membrane was stored in a glass tube in the absence of an anticoagulant. In the absence of anticoagulants, the activation of platelets would occur shortly after the blood sample comes into contact with the inner surface of the tube, leading to the initiation of the coagulation cascade. Initially, fibrinogen is localized in the upper region of the tube until it undergoes conversion by thrombin into fibrin. Theoretically, a significant number of platelets become ensnared within the fibrin network. This entrapment leads to platelet degranulation, which subsequently prompts the discharge of cytokines capable of inducing cellular migration and proliferation within the fibrin matrix. Consequently, these events initiate the initial phases of the wound healing process.[2]

According to Pezzotta et al. (2012), the use of plasma rich growth factor (PRGF) is not recommended for treating large ulcers that have already undergone neovascularization, as this can lead to the development of additional corneal opacities and an increased risk of vision loss. The degranulation of platelets during the healing process releases cytokines that promote cell migration and proliferation within the fibrin matrix, initiating the initial stages of wound healing. Previous clinical research has indicated that this biomaterial could serve as a suitable matrix for promoting healing with minimal inflammation. This novel biomaterial differs from both fibrin glue and traditional platelet concentrates. The biomicroscope examination conducted on the eighth day revealed that in the PRF graft group, re-epithelialization occurred as a smooth transition area, without any jagged edges between the PRF graft and the surrounding glasses.[13]

The utilization of the PRF graft technique offers numerous advantages in comparison to alternative methods employed for conjunctival reconstruction. PRF membrane components have the potential to enhance cellular proliferation, differentiation, and particularly cell migration in developing cells. Furthermore, the conjunctival epithelial cells are accompanied by endothelial cells that play a crucial role in neo-angiogenesis, vascularization, and the long-term viability of grafts. These endothelial cells have the ability to proliferate and migrate into the membrane, facilitating the regeneration of the damaged area. Furthermore, the gradual release of platelet cytokines occurs during the absorption of the fibrin matrix, thereby facilitating an effective healing process. Leukocytes and cytokines within the fibrin network holds significant implications for inflammatory and infectious mechanisms within the transplanted material.[6,14]

All of these processes play a crucial role in the regeneration of the ocular surface. Numerous clinical applications of platelet-rich fibrin (PRF) membranes have been documented in the fields of dental and oral surgery, periodontal regeneration surgery, management of meniscus or joint ligament tears, treatment of lower extremity ulcers, oculoplastic reconstructive surgery, and ocular surgery. The utilization of PRF membranes, which offer both mechanical and chemotactic support, renders them well-suited for reconstructive surgery. Moreover, these membranes have the capacity to improve tissue function and offer potential clinical benefits in the field of ophthalmic tissue production engineering.[2]

COLLAGEN TYPE III

The healing of conjunctival wounds is distinguished by the sequential occurrence of re-epithelialization, fibroblast cell proliferation, and extracellular matrix deposition. During the process of wound healing in the conjunctiva, fibroblast cells are generated, and a subset of these cells undergoes transformation to become myofibroblast cells. Myofibroblasts play a crucial role in the process of conjunctival wound healing. Myofibroblasts are capable of synthesizing various types of extracellular matrix proteins, including collagen. Collagen consists of three polypeptide chains referred to as alpha chains, which have the potential to assemble into either a homodimeric or

heterodimeric triple helical structure. The polypeptide chain is composed of Gly-X-Y triplets, where X and Y denote the amino acids proline and hydroxyproline, respectively. Collagen fibrils, which are cross-linked in the extracellular matrix, are formed by the intersection of three helices.[15]

Collagen can be categorized into two main groups: fibrillar (including types I, II, III, V, and XI) and non-fibrillar. Within these groups, there are further subdivisions that give rise to distinct classes. For instance, the non-fibrillar types include basement membrane types IV, as well as short-chain types VIII and X. The primary constituent of the ocular structure is collagen type I, which is accompanied by collagen types III, V, and XII. The prevailing subepithelial connective tissue is primarily composed of collagen types I and III. During the initial phases of wound healing, the synthesis of collagen type III precedes that of collagen type I, which subsequently replaces it. In addition, the presence of collagen facilitates the remodelling of the conjunctival wound and contributes to the traction necessary for the closure of the conjunctival wound.[15,16]

THE EXPRESSION OF COLLAGEN TYPE III ON PRF MEMBRANE

The initial stage of cell proliferation in the process of conjunctival wound healing occurs at the wound edge, specifically in the formation of epithelial cells. The initiation of conjunctival epithelial cell proliferation typically occurs at the periphery of the wound, typically a few hours following the occurrence of the injury. The conjunctival epithelial cells are subject to subsequent modifications, such as the cessation of intercellular exchange's heating mechanism, alterations in integrin expression, and the assembly of α-SMA, potentially resulting in increased motility. Epithelial cell proliferation is observed to commence within a timeframe of 24 to 48 hours, leading to the simultaneous occurrence of wound edge approximation.[17,18]

Granulation tissue will develop beneath the epithelial layer. The formation of this granulation tissue matrix is a result of the combined processes of angiogenesis and fibroplasia. The primary cellular entities involved in this physiological process are fibroblast cells, which typically migrate to the site of conjunctival injury within a 24-hour timeframe. Fibroblasts are responsible for the synthesis of collagen, elastin, glycosaminoglycans, and fibronectin, which collectively contribute to the formation of a loosely organized extracellular matrix. The recruitment and activation of fibroblasts, crucial for collagen production, are mediated by two profibrogenic cytokines, namely PDGF and TGF-β. These cytokines play a significant role in stimulating fibroblasts, leading to an upregulation in collagen synthesis. In addition to their primary function, fibroblasts have the ability to undergo differentiation into a distinct contractile phenotype known as myofibroblasts.[17,18]

The activated cell type exhibits distinctive features, namely the presence of intracellular α-SMA microfilaments that resemble the microfilaments found in smooth muscle cells. Myofibroblasts play a crucial role in promoting contraction and wound closure, as well as contributing to the synthesis of extracellular matrix components. Following this procedure, a preliminary underdeveloped ground network will be established. In addition, it should be noted that developing tissue in an immature state undergoes a process of remodelling, which involves the release of matrix metalloproteinases (MMPS) enzymes by fibroblasts and macrophages. This cohort of enzymes facilitates the degradation of specific components of the extracellular matrix, thereby creating a migratory route for fibroblasts within the recently developed fibrin clot and granulation tissue. The regulation of fibroblast function during their migration through newly formed tissues is also influenced by the extracellular matrix.[2]

Angiogenesis initiates promptly subsequent to the closure of conjunctival wounds. The process involves the secretion of proangiogenic factors, namely Vascular Endothelial Growth Factor (VEG) and Basic Fibroblast Growth Factor (BFGF), which are released by macrophages and platelet cells to simulate angiogenesis. The inhibition of angiogenesis in wounds can be achieved through the blockade of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (BFGF). The presence of reduced oxygen pressure and the subsequent production of lactic acid within the wound region have been found to elicit the proliferation of neovascularization.[17,18]

The initial stage of the wound healing process is marked by the localized rupture of platelets and the degranulation of mast cells. Subsequently, fibrin threads are formed, leading to the formation of blood clots. Macrophages are also involved in the inflammatory phase of tissue regeneration, wherein they secrete substantial quantities of growth factors. The number of myofibroblasts experiences an increase during the onset of the second phase (proliferation) as a result of the impact of this growth factor. This increase encompasses various processes such as angiogenesis, fibroblast cell proliferation, as well a remodelling and scar formation. Myofibroblasts are responsible for synthesizing various constituents of the extracellular matrix (ECM) and employing their contractile capabilities to facilitate the process of wound healing.[19,20]

Darby et al. (2014) stated collagen type III was observed that during the process of remodelling, which constitutes the primary constituent of granulation tissue, undergoes replacement with collagen type I. The PRF scaffold functions as a reservoir for growth factors and cytokines. The PRF scaffold is abundant in functional molecules, namely TGF-α, PDGF, and IGF-1, which play a crucial role in osteochondral repair. The bioactive molecule under investigation exhibits the ability to stimulate cellular growth and proliferation, augment the synthesis of cartilage matrix, and facilitate the expression of chondrogenic genes within the PRF scaffold.[19]

The remodelling process is predicated upon the transition from collagen type III to collagen type I. There is a notable and substantial increase in strength during the period spanning from the third to the sixth weeks following an injury. In addition, the process of re-epithelialization takes place, during which myofibroblasts within the granulation tissue persist in synthesizing matrix metalloproteinases (MMPs) and their inhibitors until the wound

healing process reaches its culmination. Upon the completion of wound healing, myofibroblasts will initiate the process of programmed cell death known as apoptosis. However, in cases where the cells within the granulation tissue fail to undergo apoptosis during the remodelling phase, there is a heightened likelihood of developing hypertrophic scars. Macrophages play a significant role in the process of remodelling by assuming a fibrinolytic phenotype, which involves the breakdown of excessive extracellular matrix (ECM) and the phagocytosis of ECM debris and apoptotic cells.[21]

The ratio of collagen type I to keloid tissue undergoes augmentation during the wound healing process. This implies that the production of collagen type I is a significant factor in the process of wound healing, and elevated levels of TGF-β stimulate the production of collagen type I. The use of PRF membranes can effectively decrease the expression of TGF-β, leading to a reduction in collagen type I production.[22]

The impact of TGF-β on conjunctival fibroblasts during the later stages of conjunctival wound healing has been well-documented. The expression of TGF-β reaches its maximum level during the inflammatory and proliferative stages of conjunctival wound healing. The migration and transition of fibroblasts to myofibroblasts can be induced by TGF-β. Transforming growth factor-beta (TGF-β) additionally augments the production of extracellular matrix components, specifically collagen type I and fibronectin. Following a conjunctival injury, quiescent fibroblasts in the vicinity of the wound undergo activation and subsequently undergo differentiation into myofibroblasts.[23] Sutyawan et al. (2013) stated the myofibroblasts have the ability to undergo proliferation and migration towards the site of injury, leading to the remodelling of the newly formed matrix and subsequent formation of scar tissue. The trans differentiation of fibroblasts into myofibroblasts plays a pivotal role in the process of conjunctival wound healing and is essential for tissue remodelling. The contractility activity of myofibroblasts is enhanced due to the presence of the α-SMA component. The augmented α-SMA component will be integrated into the actin stress fibres as a contractile apparatus to facilitate effective wound healing. Moreover, the differentiation of fibroblasts into myofibroblasts is associated with an enhanced ability to synthesize extracellular matrix proteins. The pathogenesis of conjunctival scarring is significantly influenced by key extracellular proteins, including collagen type I and fibronectin,[24,25]

The functions of collagen type I and type III are mutually supportive in the process of wound healing. Collagen type III is responsible for the initial formation of tissue, initiating the healing process. Conversely, collagen type I plays a crucial role in reinforcing and fortifying the resulting scar tissue. The achievement of an efficient wound healing process necessitates the establishment of a harmonious equilibrium in the body's production and regulation of collagen type I and type III. The primary distinction between the two entities pertains to their respective spatial positioning and distinct functional significance within the process of wound healing.[26]

Collagen type I is the principal constituent found in the connective tissue of various anatomical structures, including the skin, bones, tendons, and other dense connective tissues. The function of connective tissue in wound healing is to facilitate the formation of robust and long-lasting tissue. The formation of scar tissue occurs through the synthesis of collagen type I. This process aids in the cohesion of wound edges and imparts mechanical resilience to the affected region. Collagen type III is typically more abundant in younger or recently developed connective tissue, such as in embryonic and granulation connective tissue that forms during the wound healing process. The role of tissue remodelling in wound healing is to facilitate the initial stages of wound healing by promoting the formation of softer and more flexible tissue. The significance of this phenomenon lies in its contribution to the promotion of collagen type I synthesis and its facilitation of the reorganization of connective tissue following an injury.[27,28]

In the proliferative phase, there is a notable augmentation in the tensile strength of the wound. This enhancement can be attributed to the heightened presence of collagen type III. The phase characterized by heightened collagen deposition and subsequent wound contraction is known as the proliferative phase. This phase, occurring within the initial three weeks of the healing process, leads to a reduction in the wound's surface area. The phase of remodeling typically commences approximately 2-3 weeks subsequent to the initiation of the wound and may endure for a duration of one year or longer. The transition referred to in this stage is characterized by the maturation of collagen, specifically the conversion from collagen type III to type I until a ratio of 4:1 is achieved.[29] During the activity of fibroblasts and myofibroblasts, there is a reorganization of collagen fibres along tension lines, a reduction in wound vascularity, and wound contraction. The process of collagen maturation leads to a rise in the tensile strength of the wound, reaching its peak at 12 weeks after removal and accounting for roughly 80% of the strength observed in intact skin. The objective of this phase is to achieve optimal tensile strength by means of reorganization, degradation, and resynthesis of the extracellular matrix.[30]

The PRF membrane is capable of generating TGF-β, a potent growth factor that facilitates the transformation of fibroblasts into myofibroblasts. The expression of α-Smooth Muscle Actin by myofibroblasts leads to the deposition of this protein within the Extracellular Matrix. In addition, it will stimulate the production of collagen within the wound tissue, thereby facilitating the closure of gaps in the wound and enhancing tissue elasticity. The regulation of cellular gene expression is influenced by the interaction between connective tissue cells and the extracellular matrix. The interaction between fibroblasts and the surrounding extracellular matrix is facilitated by specific receptors known as integrins. The expression of integrins is modulated by a range of cytokines, among which TGF-β plays a significant role. The release of cytokines and growth factors that govern the expression of integrins occurs either through proteolysis from the extracellular matrix or through autocrine and paracrine

mechanisms from adjacent cells. The recognition of collagen is primarily facilitated by integrins α1ß1 and α2ß1 in fibroblasts, which are involved in the synthesis of collagen I. One specific integrin, α1ß1, regulates collagen I synthesis through a negative feedback mechanism.[31]

The disruption of the abnormal wound healing process in fibrosis cases leads to an excessive buildup of collagen within the extracellular matrix. Collagen type I is the most prevalent form of collagen present in the extracellular matrix. The upregulation of collagen I and III synthesis, coupled with a concomitant decrease in the ratio between these two types of collagen, can initiate the development of fibrotic tissue.[7,32]

THE EXPRESSION OF A-SMOOTH MUSCLE ACTIN (A-SMA) ON PRF MEMBRANE

The utilization of platelet-rich fibrin (PRF) membranes in pterygium surgery represents a novel approach that exhibits simplicity and ease of application. This technique shows promise in terms of its efficacy, as it demonstrates a lower recurrence rate compared to the use of conjunctival autografts in patients undergoing primary pterygium excision. Furthermore, the findings of the study indicate that the group receiving PRF grafts experienced minimal postoperative inflammatory reactions, tissue reactions, and granuloma formation. Multiple studies have also documented recurrence rates ranging from 8% to 39% following the use of conjunctival autograft for primary pterygium intervention.[7,33,34,35]

Tissue fibrosis is distinguished by the excessive accumulation of extracellular matrix proteins, such as collagen and fibronectin. It arises as a consequence of unregulated wound healing mechanisms in response to persistent tissue damage and inflammation. Numerous studies have demonstrated that the pathogenesis of fibrotic scar tissue in the ocular region shares similarities with fibrotic disorders observed in other vital organs, including the heart, kidney, and lung tissue fibrogenesis.[36,37] Transforming growth factor-beta (TGF-β) is involved in various stages of the ocular wound healing process, wherein it enhances the release of growth factors that facilitate cell migration, proliferation, extracellular matrix deposition, and myofibroblast formation. Elevated growth factor activity not only results in augmented rates of wound healing, but also entails an elevated susceptibility to scar formation. Hence, TGF-β emerges as a highly promising candidate for the intervention of scar tissue. Transforming growth factor-beta 1 (TGF-β1) is recognized as the primary cytokine responsible for promoting fibrosis. Upregulation of TGF-β1 expression is known to have a significant impact on the deposition of collagen and extracellular matrix, as well as the processes of wound healing and scar formation in ocular tissues. The induction of α-SMA is facilitated by TGF-β1, which has been identified as a crucial mediator.[38]

α-Smooth muscle actin (α-SMA) has been identified as a dependable indicator for the presence of myofibroblast cells. Myofibroblasts exhibit heightened contractile activity, a characteristic that is correlated with the presence of the α-SMA component. The augmented α-SMA component will be integrated into the actin stress fibres as a contractile apparatus to facilitate effective wound healing. Furthermore, fibroblasts that have undergone differentiation into myofibroblasts exhibit an augmented ability to synthesize extracellular matrix proteins. The pathogenesis of conjunctival scarring is significantly influenced by key extracellular proteins, including collagen and fibronectin.[38,39] During wound healing, fibroblasts become activated and undergo transformation into myofibroblasts, characterized by the expression of α-smooth muscle actin (α-SMA), as they migrate towards the wound site. The contractile properties of these cells are attributed to the presence of α-SMA in the microfilament bundle, which plays a crucial role in the contraction and maturation of granulation tissue.[40]

The primary distinction between PRF and PRP lies in the variation of polymerization processes, which ultimately governs their distinct biological characteristics. The process of PRP polymerization is initiated through the introduction of anticoagulants, whereas PRF polymerization occurs in a more intrinsic manner. Consequently, PRF exhibits more favorable fibrin networks for the storage of cytokines, growth factors, and facilitation of cell migration. PRF offers several advantages, including its convenient manufacturing and application processes, low cost, absence of biochemical modifications, elimination of the need for bovine thrombin or anticoagulants as activators, promotion of effective wound healing through gradual polymerization, enhanced cell proliferation and migration, supportive impact on the immune system, and facilitation of hemostasis.[41]

Dohan et al. (2006), stated that the utilization of a three-dimensional polymerized autologous fibrin matrix, which includes platelets, growth factors, cytokines, circulating stem cells, and a limited quantity of leukocytes, plays a significant role in the processes of hemostasis and wound healing. The progressive and controlled release of these factors is facilitated by their intrinsic incorporation within these scaffolds, as the fibrin netting diminishes.[42] The gradual release of autologous growth factors by PRF leads to enhanced and long-lasting effects on various cellular processes, including proliferation, differentiation, migration, and matrix synthesis. These effects are achieved through the specific binding of these growth factors to cell surface receptors.[43]

Furthermore, the platelet-rich fibrin (PRF) offers mechanical assistance to conjunctival and endothelial cells, serving as a supportive framework for their migration. The utilization of autologous platelet-rich fibrin (PRF) in conjunction with mechanical and chemotactic assistance renders it a viable option for ocular surface reconstruction, repair, and/or maintenance, along with various other clinical benefits. An additional significant discovery was that the utilization of PRF grafting led to reduced levels of postoperative inflammation, tissue reaction, and granuloma formation. Additional benefits of platelet concentrates include their autologous nature, which means they are derived from the patient's own blood. The collection process for these concentrates is straightforward, and

their manufacturing for clinical use is uncomplicated, eliminating any potential risks associated with allogenic products.[44]

The PRF group exhibited a comparatively shorter average operating time of approximately 10 minutes in comparison to the conjunctival autograft group. The implementation of conjunctival autograft involves the incorporation of supplementary surgical procedures and necessitates an extended duration. Prolonged duration of conjunctival autograft surgery not only leads to higher surgical expenses but also results in reduced levels of patient satisfaction. Moreover, augmenting the quantity of surgical manipulations and prolonging the duration of surgery can result in heightened postoperative inflammation.[45]

The technique of suturing the PRF membrane does not result in any additional damage to the conjunctival tissue and effectively preserves the normal anatomical structure of the conjunctiva. The technique of PRF exhibits a greater number of advantages in comparison to alternative methods, such as Amniotic Membrane Transplantation (AMT). PRF, when utilized as an allograft, exhibits the lowest potential for eliciting a reaction. The establishment of an AMT technique is intricate and entails a certain level of financial investment. It necessitates a profound understanding of network banking operations to mitigate the risk of inadvertent complications.[46] Furthermore, it is important to acknowledge that the potential for transmission of highly consequential pathogens, such as hepatitis B and human immunodeficiency virus, remains a constant concern that cannot be completely mitigated, despite the implementation of rigorous protocols and precautions. While it is generally believed that there is no immune response to autologous PRF, it is crucial to adhere to rigorous preparatory protocols to maintain sterility and minimize the potential for infection both prior to and following surgical procedures. The preparation of PRF is a straightforward process that can be carried out using various instruments within the confines of the operating room.[11,47]

Alpha-smooth muscle actin (α-SMA) is a specific isoform of actin that holds significant significance in the fibrogenesis process. The process of fibroblast differentiation into myofibroblasts plays a crucial role in the physiological response of wound healing and tissue repair. Apoptosis or other mechanisms of cell death contribute to the removal of blood vessels, fibroblasts, and inflammatory cells at the wound site during the maturation and remodelling phase. During the subsequent stage, fibroblasts originating from granulation tissue will undergo a phenotypic transformation, leading to the expression of smooth muscle actin, thereby assuming the characteristics of myofibroblasts.[12]

Several growth factors are produced during the wound healing process, including angiogenesis factors such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), interleukin-8, insulin-like growth factor 1, angiopoietin-1, angiopoietin-2, angiopoietin-4, epidermal growth factor (EGF), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), hepatocyte growth factor (HGF), and hypoxia-inducible factor-1 alpha (HIF-1α). Fibroblast Growth Factor has been implicated in the generation of angioblasts during the process of blood vessel development.[48]

Cell fragmentation occurs as a consequence of conjunctival excision-induced wounds. The fragment is expected to interact with the TLR-4 receptor, leading to the activation of the NF-κβ pathway and subsequent synthesis of inflammatory cytokines, namely Interleukin 6 and TNF-α. This cascade of events is likely to contribute to heightened tissue inflammation. The release of interleukin-6 induces the subsequent release of C-Reactive Protein, leading to endothelial dysfunction and activation of AT1R. This activation triggers cellular apoptosis through the ROS cascade, involving cardiolipin, caspase 8, and caspase 3. Tumor Necrosis Factor-α (TNF-α) induces the adhesion of e-selectin molecules on the endothelial cells, facilitating the binding of circulating Polymorphonuclear cells (PMNs). This process leads to the influx of PMNs into the site of injury, subsequently triggering the release of lysozyme. The action of lysozyme results in necrotic cell death. The fragmentation of conjunctival cells leads to an elevation in reactive oxygen species (ROS) levels, thereby impeding the activity of Hypoxia Inducible Factor 1 Alpha. Consequently, this inhibition of Hypoxia Inducible Factor 1 Alpha suppresses the expression of Vascular Endothelial Growth Factor (VEGF) and reduces microvascular density. The presence of Reactive Oxygen Species (ROS) leads to an upregulation of Matrix Metalloproteinase (MMP), thereby inducing degradation of the Extracellular Matrix (ECM) and subsequent reduction in collagen expression within the tissue.[31,49]

The presence of cytokines and growth factors led to the transformation of proinflammatory M1 macrophages into anti-inflammatory M2 macrophages. This phenomenon will give rise to a sequential series of events leading to the production of inflammatory cytokines, ultimately leading to a subsequent decrease in order to mitigate the inflammatory response. Transforming growth factor-beta (TGF-β) is a potent growth factor that induces the conversion of fibroblasts into myofibroblasts. Myofibroblasts are known to upregulate the expression of α-Smooth Muscle Actin, resulting in the deposition of this protein within the Extracellular Matrix. In addition, it stimulates the production of collagen within the wound tissue, thereby facilitating wound closure and enhancing tissue elasticity. The growth factor known as transforming growth factor-β (TGF-β) plays a crucial role in the pathophysiological processes associated with the formation of fibrotic tissue. The activation of transforming growth factor-ß induces the proliferation of fibroblasts and the synthesis of various extracellular matrix components, including elastin, fibronectin, collagen I, and collagen III.[31]

Svystonyuk et al (2015) stated it was determined that Fibroblast growth factor-2 plays a role in reducing the process of myofibroblast remodelling that is induced by TGF-β. The existence of a TGF-β-independent pathway in the regulation of myofibroblast differentiation. This signalling pathway was found to primarily affect collagen production rather than the expression of α-SMA. The involvement of TGF-β in the induction of fibroblast

differentiation into myofibroblasts is significant. Fibroblast growth factor (FGF) is synthesized by various cell types, including anti-inflammatory macrophages of the M2 phenotype, mast cells, T lymphocytes, and endothelial cells. The mechanism by which FGF exerts its effects involves the binding of FGF to FGFRs receptors located on the surface of specific target cells, including endothelial cells, epithelial cells, fibroblast cells, and keratinocytes. This binding event initiates a biochemical cascade, beginning with the activation of PI3-kinase. Subsequently, the Rac1 pathway is activated, which in turn regulates the activation of the JNK pathway. Ultimately, these signalling events influence the transcription of nuclear cells.[50,51]

Yun et al. (2010) stated that the phenomenon induces the proliferation, differentiation, migration of fibroblast cells, and angiogenesis. There are two distinct types of fibroblast growth factor (FGF) that are observed to be expressed, specifically those with profibrotic properties and those with antifibrotic properties. The profibrotic fibroblast growth factor (FGF) functions by promoting the proliferation and migration of fibroblasts and collagen. Conversely, the antifibrotic FGF operates by impeding and diminishing the proliferation and migration of fibroblasts and collagen through the reduction of α-SMA cell production.[52] Dolivo et al. (2017) stated the production of profibrotic and antifibrotic fibroblast growth factors (FGFs) serves as a compensatory mechanism to prevent excessive production and maintain the stability of the wound healing process.[53]

The presence of myofibroblast cells can be identified by the expression of α-SMA in tissues.[20] The synthesis of α-SMA leads to an upregulation in the generation of myofibroblasts and collagen type III, surpassing the production of collagen type I. Myofibroblasts serve as the primary effector cells in instances of fibrosis. The fibroblasts in question exhibit a phenotypic response and necessitate the expression of α-SMA, a contractile protein. Upon activation, these fibroblasts generate a substantial quantity of protein matrix.[54] Macrophages and lymphocytes are involved in the regulation of the fibrosis process through the release of different mediators that can induce the fibroblast phenotype and modulate matrix metabolism. Myofibroblasts are capable of expressing α-SMA, thereby facilitating wound contraction. The intracellular matrix will experience a process of maturation, during which the degradation of hyaluronic acid and fibronectin will occur.[55]

Ghatak et al. (2018) showed that the existence of transient cells referred to as "proto-myofibroblasts" that play a role in the conversion of fibroblasts into myofibroblasts. One notable distinction between proto-myofibroblasts and myofibroblasts lies in the lack of α-smooth muscle actin (α-SMA) in proto-myofibroblasts, wherein their stress fibres solely consist of cytoplasmic β- and γ-actin. Both of these cellular entities possess actin stress fibres and intracellular fibronectin, but they exhibit dissimilarities in terms of the fibronexus size. The fibronexus is a structural component that facilitates the transmission of forces from actin microfilaments located within the cell to the extracellular matrix of fibronectin. Proto-myofibroblasts are characterized by the presence of small fibronexi, whereas myofibroblasts exhibit larger fibronexi. Ultimately, the process of wound healing is concluded by the division and migration of epithelial and/or endothelial cells over the basal layer, leading to the regeneration of the injured tissue.[40] Meyer-ter-Vehn et.al. (2006) and Darby et.al. (2014) stated that it has been demonstrated that a decrease in mechanical stress or tissue stiffness leads to the induction of apoptosis and a reduction in α-SMA expression and contractility in myofibroblasts. The apoptosis of myofibroblasts is hypothesized to occur due to decreased levels of local growth factors that are initially involved in promoting myofibroblast viability.[19,56]

CONCLUSION

PRF, a cutting-edge biomechanical membrane technology, is very useful in ocular surface reconstruction. However, more research is needed to understand how collagen type III and α-SMA expression on the PRF membrane

ACKNOWLEDGMENTS

I thank the following individuals for their expertise and assistance throughout all aspects of our study and for their help in writing the manuscript.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

ETHICS CONSIDERATION

This literature review has followed the COPE and ICMJE protocols regarding publication ethics.

FUNDINGS.

None.

AUTHOR CONTRIBUTION

All authors have made the same contribution in writing the report on the results of this literature review.

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