

The role of Platelet Rich Fibrin (PRF) membrane and conjunctival autograft on TGF- β and collagen type 1 expression in conjunctival wound healing: a literature review



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ABSTRACT

Background: Plasma-rich fibrine (PRF) membrane is a new biomembrane useful in ocular surface reconstruction technique. Since its development, PRF membrane has been used successfully in many studies; however, some controversies maintained. This literature study aims to evaluate the role of Platelet Rich Fibrin (PRF) membrane and conjunctival autograft on TGF- β and collagen type 1 expression in conjunctival wound healing.

Methods: This literature review compiles and elaborates on previous studies from many authors to support future experimental studies, which will be conducted to compare the effect of PRF membrane and conjunctival autograft on TGF- β and type 1 collagen expression.

Results: PRF has many advantages, such as the ability to reduce inflammation, rich in growth factors, and can be a temporary scaffold for fibroblast migration. However, despite its advantages, PRF is not widely used, unlike its counterpart techniques, such as conjunctival autograft. Conjunctival autograft has long been used as an option for ocular surface reconstruction techniques, but it has some limitations. The conjunctival autograft technique cannot be performed in large or bilateral conjunctival damage. Thus, the PRF membrane technique can become a perfect solution for such a case. This literature review will provide more information on how PRF can regulate inflammation and support the conjunctival wound healing process.

Conclusion: Some impacts of PRF membrane and conjunctival autograft are still unclear, especially their effect on TGF- β and type 1 collagen expression. This literature review is expected to encourage and support future experimental studies about the impact of PRF and conjunctival autograft on TGF- β and type 1 collagen expression.

Keywords: PRF, Conjunctival Autograft, TGF- β , Type 1 Collagen, Conjunctival Wound Healing

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INTRODUCTION

Conjunctiva is an important part of the ocular surface. Healthy conjunctiva would ensure the health of another important ocular surface structure, such as the cornea. With improper treatment, wide damage to conjunctival tissue would result in eye disorders ranging from mild disease to the most severe condition, resulting in blindness. Several reasons, such as mechanical trauma, chemical trauma, postsurgical injury, and several autoimmune diseases, such as Steven Johnson Syndrome, can damage Conjunctiva.¹⁻³ There are a few techniques that are available to repair such conjunctival damage such as conjunctival autograft,

Amniotic Membrane Transplantation (AMT), oral mucosal graft, and the latest by utilizing Platelet Rich Fibrin (PRF) membrane. AMT and oral mucosal graft have several weaknesses; thus, it is not popularly performed in more rural areas with limited facilities. In areas with limited resources, conjunctival autograft is a more reliable and popular technique to repair conjunctival damage.¹⁻⁵

PRF membrane is a biomembrane processed from blood products and has been developed to the cutting edge of reconstruction technique to repair a damaged conjunctival structure. PRF Membrane contains several important growth factors and can be a scaffold that supports the conjunctival wound healing

process.²⁻⁴ One of the most important growth factors in PRF membrane is TGF- β . TGF- β is important in regulating, synthesizing, and conjunctival extracellular matrix degradation. The main component of the conjunctival extracellular matrix is type 1 collagen. An uncontrolled type 1 collagen production can result in conjunctival fibrosis. However, it is still uncertain whether there is a difference in TGF- β and type 1 collagen expression due to PRF membrane transplantation and conjunctival autograft transplantation after conjunctival excision¹⁻⁵

Based on those mentioned above, this literature review aims to elaborate and compile some knowledge from several authors and publications to understand

further the different effects of PRF and conjunctival autograft technique toward TGF- β and type 1 collagen expression. This literature review is also expected to encourage and promote more future studies in experimental or systematic review forms in the PRF and ophthalmology field.

CONJUNCTIVAL ANATOMY

The anatomy of the conjunctiva can be described macroscopically and microscopically (Figure 1). Macroscopically, the surface layers of the conjunctiva communicate with the corneal lining at the limbal margin of the cornea and the skin at the mucocutaneous junction at the margin of the lid. The conjunctiva extends from the anterior part of the superior and inferior sclera fornix to the tarsal lining of the lids and forms the conjunctival sac. Traditionally, the conjunctiva consists of three main parts: the bulbar conjunctiva, the fornix conjunctiva, and the palpebral conjunctiva.^{1,3}

The bulbar conjunctiva covers the front of the sclera and the insertions of the extraocular muscles. The conjunctiva is firmly attached to the oculi bulb near the limbus. The bulbar conjunctiva is fairly transparent in color so that the vascular tissue of the conjunctiva and the underlying episclera is sufficiently visible (Figure 1). The conjunctival fornix is divided into superior and inferior areas, interconnected at the medial and lateral canthus areas, thus forming the circular cul de sac. The conjunctival fornix is loosely attached to the skin of the superior eyelid and the rectus muscle. Within the medial fornix is a special area known as the semilunar fold (plica semilunaris), allowing eyeball movement without resistance. These structures have many goblet cells and interstitial immunocompetent cells. The caruncle is located around the medial canthus, composed of modified skin tissue with lots of vascular tissue. The caruncles also consist of sebaceous gland tissue and accessory lacrimal glands. The palpebral conjunctiva lines the inside of the eyelid lining. The palpebral conjunctiva consists of the marginal, tarsal, and orbital areas. The subepithelial connective tissue firmly

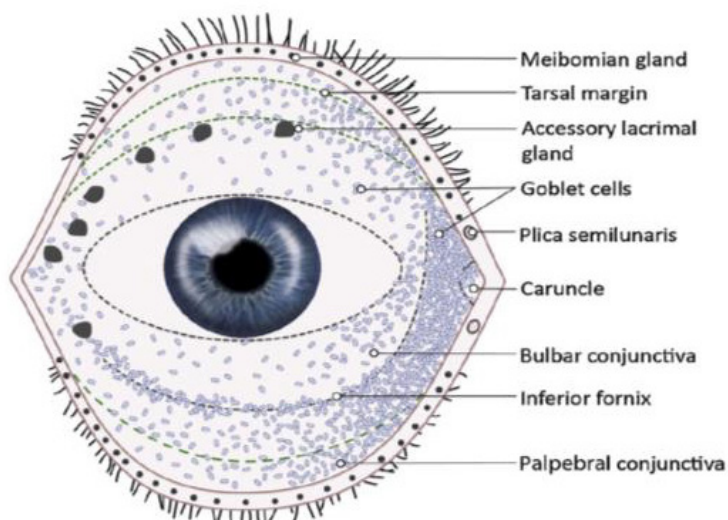


Figure 1. Regional variation of goblet cells on conjunctival surface.³

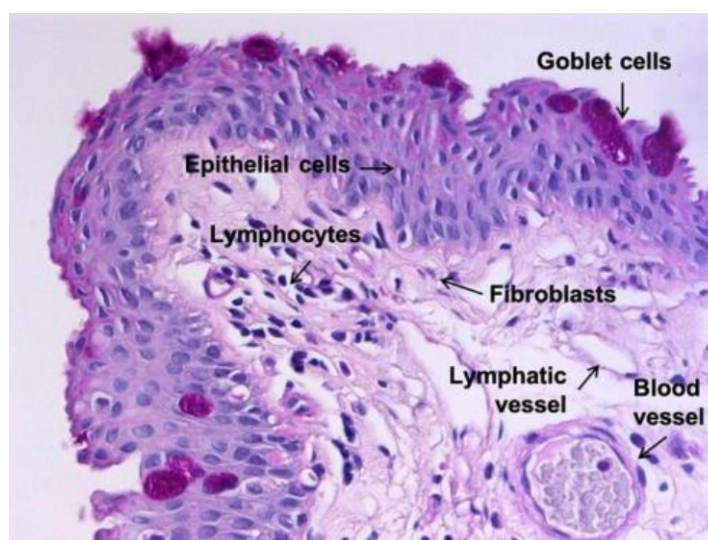


Figure 2. Histologic preparation from human conjunctival tissue with Periodic Acid-Schiff (PAS) staining. Note that the primary conjunctival cells are in the epithelial and stromal layers.¹

attaches the palpebral conjunctiva to the tarsal plate. The lacrimal punctum consists of small openings on the surface of the palpebral conjunctiva at the medial ends of the upper and lower eyelids. These structures of the lacrimal punctum support the tear drainage system. In addition, there is also a sub-tarsal sulcus structure located near the palpebral margin, which functions to trap and clean foreign objects.³

Microscopically, the surface layer of the conjunctiva consists of stratified, non-keratinized epithelium superimposed

on a rich vascular stroma layer of loose connective tissue. The structure of the conjunctival epithelium varies according to its anatomical location. Near the lid margin, the conjunctiva comprises stratified squamous epithelium without keratin, whereas the bulbar conjunctiva comprises stratified columnar epithelium without keratin.^{1,3} The conjunctival epithelium consists of two main cell types: epithelial and goblet cells. Epithelial cells are arranged in several layers and classified into basal, intermediate, and superficial epithelial cells. Between these layers are

tucked goblet cells in areas without keratin with different densities depending on their location.^{1,3} There are blood vessels, fibrous tissue, lymphatic channels, melanocytes, resident immune cells, and accessory lacrimal glands between these layers. The conjunctival epithelium is the cell type that can renew itself (self-renewing) and has a rapid rejuvenation process (turnover). The location of the conjunctival stem cell population is still controversial. Still, some evidence suggests that this population is located in the conjunctival fornix and bulbar conjunctiva lining the limbus and mucocutaneous junctions. Markers from stem cells are expressed throughout the conjunctiva, with the highest levels found in the area of the medial canthus and inferior fornix.^{1,3}

The conjunctival epithelial cells are held together mechanically by desmosome cells and are connected apically by intercellular junctions. Such a structure plays a role in resisting the stress from the environment and also as a protector from the external environment.³ Changes at these intercellular junctions lead to compromise of conjunctival integrity and are associated with the ocular surface disease. Conjunctival epithelial cells have transmembrane water channels called aquaporins. Aquaporins transfer water between the tear film's conjunctiva and the aqueous layer. The intercellular spaces between the conjunctival epithelial cells also have a role in maintaining the continuity of the movement of water molecules along the conjunctival lining.³

The conjunctiva has a rich network of blood vessels. The bulbar conjunctiva receives its main blood supply from the anterior ciliary arteries and the peripheral tarsal arcades of the lids. The vascular supply of the conjunctival fornix is similar to that of the bulbar conjunctival lining. The palpebral conjunctiva has two blood supplies, a main source from the ophthalmic artery branch and an additional supply from the facial artery branch. The anterior ciliary and peripheral conjunctival veins form the bulbar conjunctival venous drainage system, which drains into the palpebral venous plexus, leads to the palpebral venous plexus, and finally empties into the superior and inferior ophthalmic veins.

In the palpebral conjunctiva, the venous drainage system begins in the palpebral post-tarsal vein, leads to the deep branch of the anterior facial vein, and empties into the pterygoid plexus. The conjunctival lining also has an extensive network of lymphatic systems. Lymphatic drainage from the nasal bulbar conjunctiva joins the submandibular nodes, whereas lymphatic tissue from the temporal bulbar conjunctiva drains to the preauricular nodes. The medial palpebral conjunctival lymphatic tissue will be drained to the submandibular lymph nodes through the palpebral lymphatic network. In contrast, the lateral lymphatic tissue will be drained to the preauricular nodes. The lymphatic network of the conjunctival fornix is similar to that of the bulbar conjunctiva.³

CONJUNCTIVAL PHYSIOLOGY

The main function of the conjunctiva is to serve as a physical barrier against pathogens and to facilitate mucin secretion. The conjunctival tissue is immunologically active and has immune components such as toll-like receptors, which function to recognize pathogens through the recognition system of pathogenic molecular patterns. Dendritic cells can initiate and modulate ocular surface immune responses. Dendritic cells in the conjunctiva will present antigens to adaptive immune cells through pathways involving lymph nodes and/or CALT. The main humoral immune mediator in the conjunctiva is secretory Immunoglobulin A (IgA) which is produced by transformed B cells (plasma cells). Secretory IgA protects the mucosa through various actions, such as preventing microbial attachment to the conjunctival epithelium. The conjunctiva also contains endothelial venules, and specialized blood vessels that facilitate the migration of lymphoid cells, along with lymphocytes, lymphoid follicles, IgA-positive plasma cells, and associated transporter molecules. All of these components also support and strengthen the CALT function.^{1,3}

The conjunctiva has a large population of tissue lymphocytes, especially in the bulbar conjunctiva. The most abundant cell type is CD3+ T cells, most of which are CD45 (memory) T cells. In addition, the conjunctiva also contains several other

lymphocytes, such as CD8+ and CD4+ T cells. In addition, several studies have also shown the presence of several different immune cells in healthy conjunctiva, such as B cells, macrophages, plasma cells, natural killer cells (NK cells), and mast cells. These immune cells are abundant in healthy conjunctival epithelium and/or stroma. Melanocyte cells and stem cells also have an immunological role in the conjunctiva.³

CONJUNCTIVAL DEFECT RECONSTRUCTION

The conjunctiva is the largest component of the ocular surface. The conjunctiva can be damaged by various diseases or trauma, leading to scarring, tissue loss, and conjunctival dysfunction. In severe and extensive conjunctival disease or trauma, conjunctival repair may only be achieved by performing a conjunctival autograft.⁴

Several things can cause conjunctival damage, such as mechanical trauma, chemical trauma, cicatricial pemphigoid, SJS, and TEN. Small conjunctival damage can be done with a simple closure or left in a bare sclera condition. Extensive conjunctival damage must be done to close the defect with the conjunctival transplant method or AMT. In extensive and bilateral conjunctival defects, a conjunctival autograft cannot be performed because the conjunctiva of both eyes is equally damaged. Under these conditions, biological and synthetic membranes may be needed to close the conjunctival defect.⁴

There are several techniques besides AMT for closing large conjunctival defects. Some commonly used techniques are conjunctival autograft, oral mucosal graft, and nasal mucosal graft. The oral and nasal mucosal grafts are more difficult surgical techniques and special tools, so they are generally only performed by reconstruction and oculoplastic subspecialists in tertiary health centers. Therefore, a technique that is widely used and more feasible to be performed by a general ophthalmologist in the area is the conjunctival autograft technique. The conjunctival autograft technique is a surgical technique that tends to be easy to perform and can be done locally, but this technique also has weaknesses. The conjunctival autograft technique

can only be done if the defect occurs in one eye because this technique requires a conjunctival donor from a healthy neighbor's eye. Sometimes, this technique cannot be performed if trauma or ocular surface disease occurs bilaterally.^{1,4}

CONJUNCTIVAL AUTOGRAFT

Conjunctival autograft transplantation can be used in cases of conjunctival defects due to trauma, inflammation, or extensive scarring of the conjunctiva. This technique uses a conjunctival autograft to close a focal or localized defect in the conjunctiva. The most common indication for autograft conjunctival transplantation is large, recurrent pterygium. Another indication is a large pinguecula which causes chronic irritation which requires excision and transplantation conjunctival autograft to close the large defect. Sometimes in several types of surgery, such as retinal detachment surgery, strabismus surgery, and conjunctival tumor or nevus excision surgery, complications can occur in the form of shortening of the conjunctival fornix. Complications of shortening the conjunctival fornix due to this operation can be treated by performing a conjunctival autograft from a healthy neighbor's eye. Unfortunately, several diseases are bilateral, such as mucous membrane pemphigoid and Stevens-Johnson syndrome, so the conjunctiva of the neighbor's eyesight cannot be used as a conjunctival donor because they are both damaged. Oral mucosal transplantation or AMT is another alternative that can be used in these conditions.⁵

AMNIOTIC MEMBRANE TRANSPLANTATION (AMT)

AMT is one of the most frequently used therapeutic modalities to close large conjunctival defects. However, this AMT technique has several disadvantages, including the risk of disease transmission, the membrane is less transparent, the quality of the membrane varies and is unstable, and has weak mechanical strength. Other tissues that can cover conjunctival defects are conjunctival autograft, oral mucosal graft, and nasal mucosal graft.⁵

The conjunctiva is an integral part of

the ocular surface, which is important in maintaining homeostasis on the ocular surface. Conjunctival health also greatly affects the health and clarity of the cornea as an optical medium which is very important for one's vision. Several conditions, such as trauma or disease, can cause disruption or damage to the conjunctiva. The amount of damage depends on the degree of disease or trauma that occurs in the eye. Small defects in the conjunctiva can be immediately closed with simple closure sutures. However, in certain conditions where extensive defects or injuries occur, this condition does not allow simple primary closure to be carried out immediately. In very large defects, if left in a bare sclera condition, the sclera will be exposed and cause new problems, including problems with the cornea. If forced to be sewn, a strong tension will arise. In such conditions, action is needed, such as a conjunctival autograft from a healthy neighbor's eye or a membrane made through a certain process to replace the lost or damaged conjunctiva due to disease.⁵

AMT technique is the most common technique used to close large conjunctival defects. This technique utilizes processed human amniotic membranes as a material to close large defects in the conjunctiva. However, this method has several drawbacks, including disease transmission, less transparent membrane conditions, variable quality and stability of the membrane, and low mechanical strength. In developed countries, the use of AMT is indeed very reliable and has been widely used; this is made possible due to the condition of health facilities that are capable of handling and processing the amniotic membrane properly. But unfortunately, in developing countries like Indonesia, the utilization of the amniotic membrane is still low. This is because amniotic membranes are only available in tertiary health facilities in provincial capitals. Looking at Indonesia's geographical condition, the people's economy, which is still low for transportation and the lack of access to tertiary health facilities, these factors cause patients to refuse often to be referred to higher health facilities. This is a distinct weakness for using amniotic membranes

in developing countries like Indonesia, so most ophthalmologists in the regions have to use other methods to close the conjunctival defect.^{4,5}

CONJUNCTIVA WOUND HEALING

The wound on the conjunctiva will make a wound-healing process. The wound-healing process in conjunctiva consists of a series of phases that mutually overlap after the injury at the conjunctiva (e.g., operation wound, trauma, or disease). The wound or defect in conjunctiva can be caused by trauma, operation, or autoimmune disease. Conjunctival injuries can cause disruptions in the vascular and ultimately can cause leaks of cells in the blood, such as platelets, protein (fibrin), and hormones. This injury often causes vascular disorders and results in leakage of cells from inner vascular (thrombocyte or platelet), Protein (fibrin), and hormones. The first process is hemostasis, where a fibrin clot and platelet plug are formed to maintain the integrity of the vascular. The activated platelet will release a growth factor, such as PDGF, VEGF, and strong cytokines like transforming growth factor β (TGF- β) as well as interleukin.⁶

The growth and cytokine factors encourage secondary processes of conjunctival wound healing, that is, the inflammatory phase, characterized by the influx of neutrophils, monocytes, and other inflammatory cells. In some eye conditions, specific inflammatory cells and their chemical mediators play an important role that can cause fibrosis (such as eosinophil release in the case of vernal keratoconjunctivitis). Research in vitro shows that histamine plays a role in the subconjunctival fibroblast cells in patients with vernal ceratoconjunctivitis to improve proliferation, migration, and collagen production, resulting in some pro-inflammation cytokines. Phagocytic cells, such as neutrophils and monocytes, can secretly protect the proteolytic enzyme, which can cause tissue debridement. The activated phagocytes are also similar to the activated platelet, which can produce growth factors, such as fibroblast growth factor (FGF) and cytokines, such as TGF- β , which is crucial to recruit, activate, and support fibroblast.⁶

The next proliferative phase will occur, enabling granulation tissue formation under the epithelial layer. This phase features an increase in fibroblast activity. The two main keys of the process in the proliferation phase are angiogenesis, that is, the formation of new vascular and fibrogenesis, i.e., synthesis of loose tissue. The growth and critical factors are very important in this process. VEGF causes the formation of new vascular, followed by PDGF, which stimulates the ocular fibroblast. PDGF stimulates inflammatory cells and fibroblasts to release TGF- β , which will then play autocrine on fibroblasts to stimulate collagen proliferation, migration, and production. In the effect of TGF- β stimulation, Fibroblast will also differ to become myofibroblast, a contractile phenotype characterized by α -smooth muscle actin (α -SMA). Then the expression of extracellular matrix proteins will be improved to facilitate closing the injuries. Cytokines, such as interleukin-4 (IL-4) and interleukin-13 (IL-13), have increased collagen production from conjunctiva fibroblast.⁶

The final remodeling phase includes the maturation of the fibrovascular network into mature ground tissue. This final phase is characterized by Matrix Metalloproteinase (MMPs) activity, which is synthesized by the fibroblast cells, macrophages, and neutrophils. MMPs will then lead to extracellular matrix degradation, which has particular characteristics. Collagen type I will replace collagen type III and experience cross-linked and dehydration so that the peak will transform from the granulation network to a hypocultivated scar network. The decrease in the number of myofibroblasts through the process of apoptosis is important in the wound-healing phase. The elongation of myofibroblast survival is an important factor that can cause excessive scarring.⁶

The formation level or degree of scar tissue is determined by the initial damage which occurs and in response to the wound healing from the host. Controlling fibroblast activities, especially in the proliferative phase, is still an important mechanism for clinicians to control the result of conjunctival wound healing. Some fibroblast regulation points which can be regulated are: migration, proliferation,

transdifferentiation to be myofibroblast, production and collagen secretions, as well as apoptosis.^{5,6}

HEMOSTASIS PHASE

The first phase in the conjunctival wound healing process is the hemostasis phase. This phase begins immediately after the occurrence of injuries at the conjunctiva. As a wound in the conjunctival layer, the trade-blooded vessels will result in the leakage of blood cells, platelets, and plasma proteins, including fibrin.⁵⁻⁷ Local hormones such as histamine, serotonin, prostaglandin, and leukotriene will also be released in the conjunctival area. These factors can further modify vascular permeability and regulate and improve the inflammatory response process. These factors also significantly impact fibroblast activity in the final phase of wound healing. After the damage occurs in the vascular endothelial, the sub-endothelial collagen and the Von Willebrand factor will be exposed to platelets and blood clotting factors. After that, the platelet will experiment intact at the wound point through the intention of the glycoprotein reaction Ia/IIa. After the activation, platelets will play an important role in the continuous phase of conjunctival wound healing by releasing several biochemical and growth factors. Some are serotonin, thromboxane A₂, thrombin, and platelet-activating factors, forming clots and blood clotting (hemostasis). Various growth factors are also released from the activated platelet and have an important role in the next phase, that is, collagen deposition and angiogenesis deposition.⁵⁻⁷ Some growth factors, among others, are PDGF, VEGF, connective tissue growth factor (CTGF), and Insulin-Like Growth Factor (IGF-1).⁵⁻⁷

Kemokin and cytokines are also released to act as strong inflammatory immolations. Those cytokines are the TGF β 1 and β 2, IL1, IL8, Macrophage inflammatory protein 1 α (MIP-1 α), and the other factors. The most dominant factor is platelet factor 4 (PF 4), which acts as a protein of immolates for monocytes and neutrophils and plays a role in immunoregulator activity. Simultaneously, the freezing factors will be activated and further enter into complex processes,

ultimately leading to fibrinogen conversion to fibrin mediated by thrombin. The fibrin matrix will then be stabilized through the cross-linking process on the platelet plug to achieve progressive hemostasis and form a stable clot.⁵⁻⁷

INFLAMMATORY PHASE

The presence of neutrophil and monocyte cells characterizes the inflammatory phase. These cells act as early in response (Early Responder), which migrates to the location of the occurrence of the wound through the recruitment process of various cytokines. Neutrophils can be found a few minutes after the conjunctival injury and generally will reach peak levels within 48 hours. Neutrophils can penetrate the network by releasing proteolytic enzymes such as local collagen and elastases to phagocytes bacteria and extracellular debris. Monocytes in circulation and local monocyte will further experience differentiation to become macrophage networks, mostly supported by TGF- β cytokines. The activated macrophages help contribute to the processing network and phagocytosis. In addition to that, they also have an important role in regulating or controlling the conjunctival wound healing process. This process can occur by releasing various cytokines and growth factors added by the interaction with lymphocytes and other fibroblasts. The growth factors of the macrophage in the network include PDGF, TGF- β , epidermal growth factor (EGF), and fibroblast growth factor (FGF). Macrophages are also very important to activate fibroblast in an adequate amount in doing the maintenance. In wounds with a small number of macrophages, the debris will be settled, and there will be a decrease in growth factors, as well as slow motion production and granulation tissue maturation. The other important inflammatory components, such as lymphocytes, will generally appear on the conjunctival wound after the 5th day and are known to double function in regulating conjunctival wound healing. In the early period of inflammation, the T lymphocyte cells will stimulate the fibroblast, macrophages, and endothelial cell function, but in the next phase will even lower their activities and production

(downregulation). The cytokines released from the T-lymphocytes cells include PDGF, TGF- β , IL-4, and interferon γ .⁵⁻⁷

PROLIFERATION PHASE

The most superficial location of cell proliferation in conjunctival wound healing is the epithelial formation on the wounded edge. Conjunctiva epithelial cell proliferation generally begins on the edge of the wound after several hours after the occurrence of the injuries. The conjunctival epithelial cells will further modify, including the loss of the heating mechanism of interchanges among cells, integrin expression changes, and assembly of the α -filament smooth muscle actin (α -SMA), which can cause more motile. Within 24 to 48 hours, the proliferation of epithelial cells will start side by side so that the wound's edges will experience reapproximation.⁵⁻⁷

Under the epithelial layer, the granulation network will be formed. The matrix of this granulatory network is formed from the combination of angiogenesis and fibroplasia. The main cell that plays a role in this process is the fibroblast cell, which will generally reach the location of the conjunctiva wound within 24 hours. Fibroblasts will synthesize collagen, elastin, glycosaminoglycan, and fibronectin to form an extracellular matrix of loose tissue. Two profibrogenic cytokines responsible for recruiting and activating fibroblasts are PDGF and TGF- β , which will stimulate the other fibroblast to increase collagen production. Fibroblast will also differ to be the other contractile form called myofibroblast.⁵⁻⁷ This activated cell type is characterized by an intracellular microfilaments series of α -SMA, similar to plain muscle cells. Myofibroblast will help facilitate contractions and closing injuries and contribute to extracellular matrix production. After the process, the starting ground network will be formed, which is still immature. Furthermore, the immature grow tissue will experience remodeling by releasing enzymes matrix metalloproteinases (MMPS) from fibroblasts and macrophages. This enzyme group will help degrade some extracellular matrix to form a path for fibroblast migration through the fibrin clot and newly formed granulated network. This

extracellular matrix also regulates the fibroblast function when migrating through a newly formed network.⁵⁻⁷

The angiogenesis process will also begin as soon as the conjunctival wound closing process occurs. Proangiogenic factors that play a role in this process are VEG and Basic Fibroblast Growth Factor (BFGF), which both are secreted from macrophages and platelet cells to imitate that process. The blockade of VEGF and BFGF can cause total inhibition of angiogenesis in wounds. Low oxygen pressure and the formation of lactic acid in the wound area will also stimulate the growth of new blood vessels.⁵⁻⁷

THE REMODELING PHASE

In the final phase of wound healing, the immature fibrovascular network will be over hared and experience remodeling to become a mature scarring network. Plasminogen and MMPs activators will be extracellular matrix degradation mediators by eliminating the hyaluronan and fibronectin layers. It has been known that there are as many as 20 types of MMPs. Each MMP has a bond affinity and specific interaction with inhibitors of metalloproteinase networks, which will block the MMP activity. Some inflammatory cells (including fibroblasts, neutrophils, and macrophages) can synthesize MMPs in the conjunctival wound location.⁵⁻⁷ Proteoglycan will be dedicated and type III collagen replaced by type I collagen, along with the decline in fibroblast. For a few weeks up to several months, the collagen will then experience cross-tie (cross-linked) and decrease water content (dehydration) to increase the strength and pull of collagen networks. Consequently, the extracellular matrix will experience transition changes from loose granulated tissue and has a lot of cells to become solid scar tissue and a small cell (hypocellular). A crucial process in this phase is the occurrence of death cell fibroblast through apoptosis. The trigger of the apoptosis is still not known for certain, but there is a hypothesis that states that this is due to mechanical stress on the network. In this case, time accuracy is the key because early apoptosis can cause the closure of wounds that are not adequate. The decreased apoptosis from fibroblasts

can cause scar tissue and excessive fibrosis.⁵⁻⁷ Most normal grate tissue will show low fibroblast activity after one year of the formation. Biopsy of the ground-planted ground tissue in humans indicates that fibroblast activity is persistent for up to 10 years. Mitomycin C (MMC) has shown the effect of apoptosis on fibroblast invitro, and this is a possible mechanism that can be intervened to modulate injuries. Although classified as a different and striking stage, the remodeling phase can occur simultaneously and overlap with other wound healing phases. This is because phase remodeling starts a few days after the injury and can occur until several weeks. The modulation process of this remodeling phase is not easy to understand, especially about the time when the mediators exist which play a role in this phase.⁵⁻⁷

CONJUNCTIVAL FIBROSIS

Modulation in the conjunctival wound healing process has the potential to improve the result of conjunctival healing in such eye diseases, such as ocularcircular pemphigoid keratoconjunctivitis, vernal, pterygium, and post-operation wounds such as trabeculectomy. On one side, anti-inflammatory and antimetabolite drugs have been widely used and provided success, but the use of these substances is inseparable from various complications. Research to find more specific substances to control conjunctival fibrosis with minimal side effects until now is still appealing to researchers, especially in the field of eyes. Therefore, there are currently many types of research focusing topics on fibroblast proliferation regulation, differentiation post-inflammation cells, extracellular matrix production, and cell apoptosis in the wound healing process.⁵⁻⁷

The growth and cytokine factors will spur inflammation, a secondary part of the wound healing process, marked by the neutrophil influx, monocyte, and other inflammatory cells. In some eye diseases and post-trauma, specific inflammatory cells and chemical mediators are crucial in creating fibrosis (histamine release in keratoconjunctivitis vernal disease). Invitro studies show that histamines have a role in the fibroblast conjunctiva of patients with vernal keratoconjunctivitis

to improve proliferation, migration, increase collagen production, and trigger the release of some pro-inflammation cytokines.⁵⁻⁷ Phagocytic cells, such as neutrophils and monocytes, can secrete proteolytic enzymes and trigger the occurrence of network debridement. Like an activated platelet, activated phagocytes also stimulate the production of factor growth, such as the Fibroblast Growth Factor and cytokines, such as TGF- β , which are essential to recruit, activate, and maintain the fibroblast cells.⁵⁻⁷

In the hemostasis phase, inflammatory phase, and proliferation phase, there will be an increase in TGF- β levels, where the most drastic increase will occur in the inflammatory inflammation phase. A study researching fibroblast in patients with ocular cicatricial pemphigoid demonstrates that increasing TGF- β levels will stimulate expressions from connective tissue growth factor (CTGF). CTGF is an important mediator that triggers an increase in fibroblast activity. CTGF is mostly found in the networks considered active fibrosis process and will experience excessive expressions such as on post-operation trabeculectomy on the rabbit model. In some studies, CTGF shows the effect of stimulation on Tenon capsular fibroblast cells to proliferate and differentiate to become myofibroblasts and improve type 1 collagen and fibronectin. Some other studies show that obstacles or inhibitions on some TGF- β axis components will potentially control fibrosis.⁵⁻⁷

PLATELET CONCENTRATE

Platelet concentrates are generally used in trauma wounds, surgery wounds, or some other diseases to improve the quality and accelerate wound healing. In all types of injuries, blood experiencing coagulation forming fibrine and the matrix is the first step of natural wound healing. Platelet concentrates are designed to strengthen the natural wound healing process like fibrin glue, which has been used for more than 40 years as a surgical adjuvant to improve wound healing quality. As time passes, the optimum concentration of wound healing has evolved into a more complicated concept from network regeneration supported by the growth

factors and cells in it. Initially, though, it was regarded/considered as a surgical adjuvant, but lately, due to the progress of research, platelet concentrates, or PRP, has been much-claimed/stated as one of the main instruments in the main strategy of regenerative medicine.⁵⁻⁷

MEMBRANE PLATELET RICH FIBRIN (PRF)

Membrane platelet-rich fibrin (PRF) is the second generation of platelet concentrate, which was first described by previous studies.⁵⁻⁷ PRF membrane comprises a 3-dimensional (3d) polymerized autologous fibrin matrix incorporated from platelet concentrates, growth factors, cytokines, circulating stem cells, and some leukocytes, which have chemotactic functions in the injury healing process. In addition, the macro and micro architecture of the membrane RFF provide the function of a temporary scaffold to make the growing new cells migrate easily to the surface of this membrane. This temporary scaffold is analogous to a buffer pole used as a place for crawling plants. In this context, the PRF is analogous to a buffer pole and conjunctival cells, regarded as a crawling plant. The combination of mechanical and chemotactic benefits makes the PRF membrane suitable for reconstructing, fixing, or maintaining the ocular surface's stability to provide potential and clinical benefits for corneal or conjunctival operations. Membrane PRF has been widely used in other health areas, such as dental and mouth fields, throat, nose ear (THT), Orthopedic, and plastic surgery/operations.⁵⁻⁷

The PRF membrane produces many growth factors, covering PDGF, VEGF, and TGF- β , which are released by the PRF membrane from 7 to 28 days. In addition, the PRF membrane also produces matrix protein, such as thrombospondin-1, fibronectin, and vitronectin which has a key role in hemostasis and conjunctival wound healing. Besides, the PRF membrane can be a scaffold or a bridge that gives mechanical support for new cells to proliferate, differentiate, and migrate. All of these processes are very important for the regeneration ocular surface. Some clinical applications of the PRF membrane have been reported on operations in the

field of dental and mouth, periodontal regeneration operations, therapy on the risk of the meniscus or joint ligaments, treatment in inferior extremity ulcers, occlicoplasty reconstruction operations, and operations in the eye field. The combination of mechanical and kemotactical support produced by the PRF membrane makes it suitable and appropriate for reconstruction operations, improving network functions, and giving potential clinical benefits for network production engineering applications in the field of eye.⁵⁻⁷

THE PREPARATION AND PROCESS OF PRF MEMBRANE

To create the autologous PRF membrane, 5 ml of fresh blood sample is taken from the rabbit femoral vein and put into a glass tube without anticoagulant. This process is done using a common anesthetic procedure in rabbits. The blood sample is then centrifuged with the 2700 rotary per minute (RPM) for 12 minutes using centrifugation.⁵⁻⁷ After the centrifugation, the fibrin clot is concentrated among the corpuscle of red blood cells in the base of the tube and the acellular plasma liquid (commonly termed platelet-poor plasma or PPP) at the top of the tube. PPP is then taken by pipette in the supernatant section of the destined type of blood sample. After the PPP is discarded, the fibrin clot is separated mechanically from red blood cells using force and then leveled slowly using a PRF membrane box made on a custom to absorb the liquid. After these steps are performed, the PRF membrane will form and be ready to close the defective bare sclera. The PRF membrane will be lit in the conjunctions around the bare sclera with the absorbable thread 7.0. In order to fix the PRF membrane, it takes approximately 4 stitches on the four membranes by applying interrupted stitching techniques, 2 stitches in the superior part and the other 2 stitches in the inferior part. Then, after the operation (Post-operation), Moxifloxacin 0.5% (Vigamox) is given 4 times a day for as many as 10 days and a follow-up is also done to evaluate the existence of secondary infections, sclera necrosis, symblepharon, or retraction in the conjunctival fornix area.⁵⁻⁷

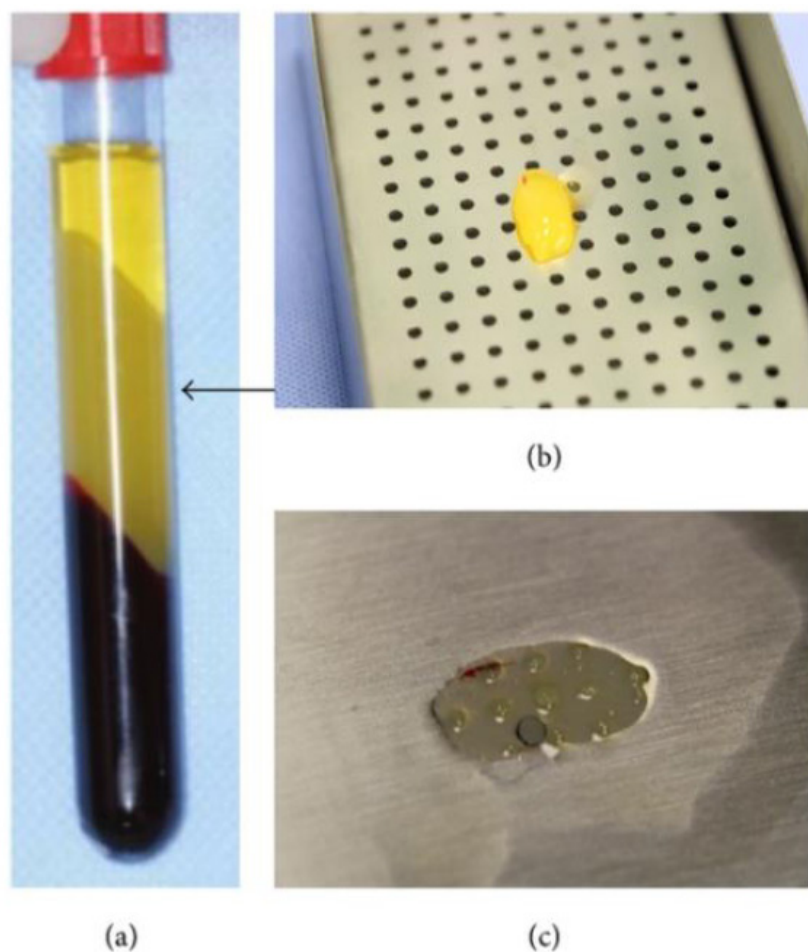


Figure 3. The procedure to make PRF membrane. (a) After the blood sample was centrifuged, three layers were formed. The middle layer consists of a fibrin clot that is then separated from the supernatants, which consist of acellular plasma liquid. The fibrin clot is then mechanically separated with the lower layer of red blood cells. (b) After the separation, the fibrin clot is pressed on the PRF box. (c) After the fibrin clot is pushed, it will become a flat PRF membrane ready for the conjunctival reconstruction procedure.⁵

ARCHITECTURE AND PRF MEMBRANE COMPOSITION

PRF clot, obtained after centrifugation, contains most platelets and leukocytes from the whole sample Whole Blood. The platelet and leukocyte distribution follows a three-dimensional pattern caused by gravity forces during centrifugation. In the PRF clot, it is estimated that there are 97% platelet and > 50% leukocytes from all Whole Blood samples. Platelet and fibrin form a large coagulation cluster on the membrane to several millimeters above the red blood cell layer. Fibrin networks formed on membranes are very mature and have high density, so that

they can be used as biomaterial materials. Understanding the PRF architecture clot is important to maximize the use of PRF membrane in various clinical situations. Research shows that the type of tube used to make PRF (glass tube or plastic tube with a glass layer in the inside part) equally gives the final result of similar PRF biomaterial architecture.⁸

PRF MEMBRANE CLINICAL APPLICATION IN EYES

PRF has been widely used recently in the eye field to close the conjunctivation defect after Pterygium post-operation. In 2021, Yang et al. reported the research results of

108 pterygial excision patients (108 eye patients).⁹ The total number of the patients (108 patients) were divided into 2 groups; in which the first group of 56 eye patients was treated by pterygium excision followed by limbal conjunctive autograft (LCA) and the other group consisting of 52 eye patients was treated by pterygium excision with the given membrane PRF. The PRF membrane is produced using the patient's blood sample, followed by centrifugation.⁹ The average or the mean time operation is significantly faster in the second group, the patient group with excision and PRF membrane. Recurrence occurs in 2 cases conducted through LCA (3.6%), while there is no recurrent, which is reported on the PRF membrane. There is no graft loss reported in both group. Moreover, there is no complication in intra or post-operation. Yang et al concluded that using PRF membrane in post-terygium excision is a simple and easy method. This is more convenient for patients and operators as well.⁹ In addition, giving PRF membrane also takes shorter operation in terms of time, lower recurrence rate, safe, and minimal complications so that it can be widely used in pterygium treatment.⁹

NEW ZEALAND WHITE RABBIT (ORYCTOLELAGUS CUNICULUS)

Theoretically, *Oryctolagus cuniculus* or the New Zealand rabbits, are not species often used for experimental research in the field of ophthalmology. The rabbit's eyeball is quite prominent. In adult rabbits, the average flat size of the eyeball is 18 mm horizontally, 17 mm vertically, and has axial anterior and posterior 16 mm in length. The use of rabbits as trial animals in experimental studies to examine vision/eye function is limited in some literature.¹⁰

In ophthalmology research, the rabbit's eyes have a lower similarity than the trial animal primary species. The primary species, such as monkeys, have eyes with more physio anatomy similar to humans. However, for researchers in the field of ophthalmology, rabbits have some advantages, which are easily got, more economical in terms of price, cheaper in cost, easier to control, and have larger eyeballs rather than mice species. In this study, the researcher did not choose mice due to the small diameter of the eyeball.

In contrast, the researcher needed to make incisions injured at the conjunctiva with certain diameters. Using mice in animal trials will make treatment more difficult and increase the risk of treatment error.¹⁰

The conjunctiva of the rabbit is divided into two parts: bulbs conjunctiva and palpebra conjunctiva. Overall, the total conjunctival area of rabbits reached 50% of the total area of human conjunctiva. Palpebra conjunctiva on rabbits stuck strongly on the palpebra and has an average thickness of 40 micrometers. In the fornix area, many goblet cells and intrapetite glands are scattered among epithelial cells.¹⁰ The bulbs' conjunctiva is thinner, about 10-30 micrometers, with fewer goblet cells, where the total number will increase in conjunctivas near the limbus. The rabbit conjunctiva has plasma cells containing immunoglobulin A (Ig A) and tends to have large and inflammable cells in the conjunctiva in response to inflammatory conditions. This is a benefit because the research is intended to know the immune response at post-inflammatory conjunctiva, which is easier to do.¹⁰

THE ROLE OF TYPE 1 COLLAGEN

The reepithelialization process, fibroblast cell proliferation and extracellular matrix deposition characterize conjunctiva wound healing. Fibroblast cells formed during the conjunctival wound healing process will experience modification (some) to be myofibroblast cells. In the conjunctival wound healing process, myofibroblast has a significant role. Myofibroblast will synthesize some extracellular matrix proteins, such as type 1 collagen. Type 1 collagen has the most protein in the corneal and conjunctive stroma. The type of collagen 1 will then help in the conjunctival remodeling process and provide a pulling power that helps close the conjunctival wound.¹¹

Type 1 collagen is a major component in the extracellular matrix, which maintains network integrity. Several studies show that the keloid network's type 1 collagen ratio has increased, indicating that type 1 collagen production has an important role in the wound healing process. The increased level of TGF- β triggers type 1 collagen production. The research

shows that providing a substance that presses TGF- β levels will decrease type 1 collagen.¹²

THE ROLE OF TRANSFORMING GROWTH FACTOR-B (TGF-B)

TGF- β has two important roles in the synthesis matrix regulation and degradation. TGF- β can act as \autocrine in the biological process in vivo and in vitro. Establishing TGF- β occurs in the endoplasmic reticulum with the following mechanism. Initially, preprotein that experienced a proteolytic process formed two TGF- β monomers through disulfide bridges to be a pro-TGF- β dimer by furin convertase enzymes. It is split (cleaved) into small latent TGF- β complex (SLC), which is noncovalent with latency-associated peptide (LAP) and large latent TGF- β binding protein (LTBP) form a large latent complex (LLC). The LLC will then be secreted to the extracellular region by the influence of reactive oxide species (ROS), integrin and pH acid, plasmin, plasminogen activator, thrombin, MMPS and elastase.^{13,14}

The research shows that the formation of fibrotic scar tissue in the eye has a mechanism similar to fibrotic diseases in other organs such as the heart, kidney, and lungs. In this case, TGF- β is known to be the main contributor to the scarring of the scar-fibrotic in the eye organ. In the process of fibrosis, TGF- β will trigger fibroblast cells so that there will be excessive production of extracellular matrix proteins such as collagen and fibronectin. Therefore, by supplying the TGF β , the process of fibrotic scar tissue on the conjunctiva will be prevented.¹³⁻¹⁵

TGF- β affects fibroblast conjunctiva in the advanced phase of the conjunctival wound healing process. TGF- β peaks on the inflammatory phase and proliferation in the conjunctival wound healing. TGF- β can induce migration and transition fibroblast to myofibroblast. TGF- β also increases the synthesis of extracellular matrix (type 1 collagen and fibronectin). After the conjunctival injury, the fibroblast, which is inactive around the wound, will be activated and differentiated into myofibroblast. These myofibroblasts will proliferate and migrate to the wound area and can cause remodeling

in the new matrix so that scar tissue is formed. Fibroblast transdifferentiation to myofibroblast is a crucial phase in the conjunctival wound healing process and is needed for remodeling the network. Myofibroblast shows an increase in contractility activity associated with the existence of α -SMA components. The increased α -SMA components will be incorporated into actin stress fibers as a contractile apparatus to ensure good wound healing. In addition, fibroblast, which has been differentiated as myofibroblast, also increases extracellular matrix protein production capacity. The main extracellular protein, such as type 1 collagen and fibronectin, has been known to have a big role in the pathogenesis of the conjunctival scar network.¹³⁻¹⁵

This literature study has elaborated and compiled some knowledge on using PRF membrane as an alternative for conjunctival autograft in conjunctival reconstruction. However, some questions regarding the significance and different outcome between PRF and conjunctival autograft toward TGF- β and type 1 collagen expression is still uncertain and need to be confirmed in future studies.

CONCLUSION

PRF is the latest cutting-edge technology as a biomechanical membrane which is very useful in ocular surface reconstruction. However, further research is still needed to understand the impact of PRF membrane on TGF- β and type 1 collagen expression.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

ETHICS CONSIDERATION

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AUTHOR CONTRIBUTION

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

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REFERENCES

- Diebold Y, García-Posadas L. Is the Conjunctiva a Potential Target for Advanced Therapy Medicinal Products?. *Pharmaceutics*. 2021;13(8):1140.
- Kim DJ, Jung MY, Pak HJ, Park JH, Kim M, Chuck RS, et al. Development of a novel hyaluronic acid membrane for the treatment of ocular surface diseases. *Sci Rep*. 2021;11(1):2351.
- Downie LE, Bandlitz S, Bergmanson JPG, Craig JP, Dutta D, Maldonado-Codina C, et al. CLEAR - Anatomy and physiology of the anterior eye. *Cont Lens Anterior Eye*. 2021;44(2):132-156.
- Makuloluwa AK, Hamill KJ, Rauz S, Bosworth L, Haneef A, Romano V, et al. Biological tissues and components, and synthetic substrates for conjunctival cell transplantation. *Ocul Surf*. 2021;22:15-26.
- Can ME, Çakmak HB, Dereli Can G, Ünverdi H, Toklu Y, Hücemenoğlu S. A Novel Technique for Conjunctivoplasty in a Rabbit Model: Platelet-Rich Fibrin Membrane Grafting. *J Ophthalmol*. 2016;2016:1965720.
- Zada M, Pattamatta U, White A. Modulation of Fibroblasts in Conjunctival Wound Healing. *Ophthalmology*. 2018;125(2):179-192.
- Seibold LK, Sherwood MB, Kahook MY. Wound modulation after filtration surgery. *Surv Ophthalmol*. 2012;57(6):530-550.
- Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J*. 2014;4(1):3-9.
- Yang N, Xing Y, Zhao Q, Zeng S, Yang J, Du L. Application of platelet-rich fibrin grafts following pterygium excision. *Int J Clin Pract*. 2021;75(10):e14560.
- Peiffer RL, Pohm-Thorsen L, Corcoran K. *Models in Ophthalmology and Vision Research*. Second Edi. The Biology of the Laboratory Rabbit. Second Edition. Academic Press: Inc. 1994:409-433.
- Witt J, Borrelli M, Mertsch S, Geerling G, Spaniol K, Schrader S. Evaluation of Plastic-Compressed Collagen for Conjunctival Repair in a Rabbit Model. *Tissue Eng Part A*. 2019;25(15-16):1084-1095.
- Yonemura H, Futakuchi A, Inoue-Mochita M, Fujimoto T, Takahashi E, Tanihara H, et al. DNA methyltransferase inhibitor suppresses fibrogenetic changes in human conjunctival fibroblasts. *Mol Vis*. 2019;25:382-390.
- Nababan AR, Suryowati DI, Komaratih E, Primitasari Y, Legowo D, Notopuro PB, et al. Platelet-rich fibrin (PRF) graft and amniotic membrane graft on transforming growth factor- β (TGF- β) and type 1 collagen post conjunctival excision. *Bali Medical Journal*. 2022;11(3):1744-1750.
- Aswati N, Indriaswati L, Sutjipto, Fauziah D, Utomo B. The effect of fibrin glue on suppressing Transforming Growth Factor- β (TGF- β) expression compared to Mitomycin-C (MMC) and Triamcinolone Acetonide (TCA) as antifibrotic agents in contracted socket prevention. *Bali Medical Journal*. 2022;11(3):1503-1508.
- Sutyawan E, Niti S, Widianara R. Effect of conjunctiva-limbus transplantation with fibrin glue compare to suture technique on stability of the graft attachment in pterygium surgery. *Bali Medical Journal*. 2013;2(1):27-32.



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