RESEARCH ARTICLE

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Role of Growth Factors-Rich Plasma, Gel and Membrane in Dermal Wound Healing and Injured Tissue Restoration and Regeneration

Tariq Mehmood Dar and Kashif Ali Samin

ABSTRACT

***Background*: The socioeconomic burden on society grows as the incidences of chronic age-related degenerative diseases increase which demand extensive wound care as well. To speed up the healing of cutaneous wounds, new wound healing treatments must be researched, trialed & developed. Regeneration therapies are gaining popularity since they are less invasive than other treatments.**

***Method*: Published research paper have been reviewed to develop a concept and analyze the role of Platelet-rich plasma (PRP) and Growth factors-rich plasma in speedy wound healing and tissue regeneration. Three patients with diabetic ulcers have been selected and applied Growth factors-rich plasma and membrane treatment on weekly basis and analyzed the results.**

***Results*: Growth factors-rich plasma injection and membrane application on wound have produced remarkable wound healing outcome within 3 to 6 applications with new vascularization and re-epithelialization.**

***Conclusion*: Growth factors-rich plasma and membrane application on wound gained favor as a wound-healing therapy due to its constituents which have remarkable potential to speed up the injured tissue repair and regeneration. The release of cytokines with platelet-derived growth molecules enveloped in alpha-granule, promote & facilitate wound healing.**

Keywords: Alpha-granule, degenerative diseases, growth factors-rich plasma, platelet-derived growth factors, wound healing process.

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I. Introduction

In vertebrates, the body's largest organ is the skin, accounting for roughly ten percent of total body mass [1], [2]. Its capacity to self-repair and renew and acting as a barrier between the exterior and internal environments, is crucial for defense and survival [3]. Anatomical structural disruption and loss of skin functional integrity are two characteristics of a wound or lesion [4]. The interplay of various cells, growth proteins, and molecules in wound healing mechanism is a vigorous activity [5], [6]. The disruption of this mechanism can result in chronic inflammatory arrest, which can lead to the development of long-standing complicated wounds or the formation of extreme granular substance [7].

In recent years, the number of people suffering from degenerative diseases related to aging has increased significantly, placing a significant biological strain on chronic wounds. Surgery, burns, infections, pressure ulcers, diabetic and venous ulcers all contribute to the development of acute and chronic wounds in millions of individuals across the world [4]. Chronic lesions in the United States cost $25 billion each year [8]. Around 15 percent of the almost 150 million diabetics globally are affected by complicated foot ulcers [9]. It is estimated that chronic wound care accounts for around 2 percent of total European health expenditures [10]. Study revealed that chronic wound treatment is just 50 percent effective and is also extremely expensive because continuous and uninterrupted treatment is required [11].

Even with considerable advancements in medical treatment and nutrition, new dermal wound healing approaches are necessary. The medical processes are rapidly progressing toward the invention of minimally invasive or non-invasive treatments and accelerated therapies that will reduce morbidity and improve our patients' quality of life through functional rehabilitation. These simple and low-cost treatments may have recently helped to lower the cost of conventional general health care [12]. Regenerative medicine is a relatively evolving field of medical investigation which targets to repair, restore, and substitute wounded cutaneous substance [13].

Tissue repair can be improved and expedited using plasma enriched with platelet growth factors, which is an autologous

therapeutic technique [14]. Autologous plasma with platelets is a biological product derived from the patient's own blood (after centrifugation process) with a greater platelet concentration within an obtained plasma fraction compared to normal blood [15]. Platelets are essential in wound healing science because of their regenerative activity through restorative proteins and repair substances [16].

The US FDA and the European Medicines Agency are authorized PDGF for application in wound patients thus far [17], [18]. Alpha granule substances of platelet are polypeptide molecules that are soluble and diffusible which contribute to the differentiation, proliferation, and metabolism of a wide range of cell types [14]-[19]. There is evidence to suggest that they participate in regeneration of endothelium and epithelial cells, angiogenesis, collagen production, and soft tissue repair [20].

It has been known since the 1940s that growth factors can be used to enhance cutaneous wound healing by topical or intralesional administration or by applying growth factors-rich membrane over wound [21]. A number of clinical trials have demonstrated that growth factors-rich plasma is helpful in the treatment of inflammatory dermal wounds [22], [23], skin injuries [24], [25], burn related wounds [26], and in aesthetic applications [27], [28].

Even though there are several therapeutic procedures for improving wound healing, the clinical application of new therapies continues to be a challenge. For the time being, researchers are still searching for regenerative treatments that will alleviate the strain on healthcare systems. The clinical and scientific findings of cutaneous wound regenerative treatments such as growth factors-rich plasma and stem cells, both in vivo and in vitro, have been encouraging. Because of the reasons indicated above, this paper will focus on the regenerative field of cutaneous wound healing, with a particular emphasis on growth factors-rich plasma as an effective and autologous therapeutic option for this condition.

A. Healing Phases – Wound

A sound knowledge of the pathophysiology of wound healing stages demonstrates in the form of better clinical outcome [25]. In wound healing, body establishes numerous intracellular and intercellular communication channels within a variety of cell types where various physical, chemical, and cellular factors have been involved in this biological process [5], [29].

Wound regeneration attempts to restore morphological and functional aspects of wounded tissues having a perfect replica of the normal one, whereas repair of the wound involves producing tissue over wound having lesser individualities equated with the normal one [30], [31].

Wound healing involves distinct phases, including hemostasis/inflammation, cellular evolution & renovation/remodeling, controlled through a number of growth proteins and cytokines directly or indirectly [25], [32], [33].

Initially, skin damage stimulates many physiological functions to appear. One of them is stimulation of platelets to rush at the point of injury, aggregate there and form fibrin clot to produce hemostasis, and also providing chemotaxis of various cells at the point of wound [34], [35]. Several growth proteins along with cytokines are released in this 1st phase [24]. The presence of blood clots and platelet degranulation indicates the presence of inflammation. The emission of serotonin, growth proteins and histamine provide chemotaxis of white blood cells (WBCs) from the capillaries to the site of injury by enhancing the permeability of capillaries [36]. During the first one to two days following an injury, neutrophils are at their highest concentration, and they aid in the prevention of infection through interactions with macrophages, and they also facilitate in the activation of keratinocytes, fibroblasts, and immune cells [34]. When the phase marked as inflammatory over, macrophages excrete TGF-beta, IL, and TNF [5]. These proliferative substances provoke the proliferative phase which remarkably known for angiogenesis and re-epithelialization with new matrix synthesis [31]. VEGF stimulates endothelial cell migration; it also attracts fibroblast from adjacent non-wounded tissues and results in the formation of new blood vessels [37]. Fibroblasts with activation are helpful in the production of interstitial substances which are rich in immature type III collagen in comparison to mature type I collagen present in regular skin [34], [38]. Almost 21 days after the original lesion, the wound has reached its peak collagen concentration [25]. After 3-5 days, granulation tissue replaces fibrin plug and injured tissues start to contract [35]. At last, the final phase of wound healing called as maturation phase or also known as remodeling appears, where cellular loss is noted at the injured site due to migration and apoptosis [39, 40]. In the course of the remodeling process, collagens create strong association with different protein substances, boosting stretching power by up to 80 percent equated with non-injured skin [34]. Studies have shown that the ratio of immature type III collagen to mature type I collagen decreases with time, which takes around 2 years to convert immature collagen into mature one [34], [39].

A wide range of growth proteins, cytokines, intercellular adhesion molecules, metalloproteinases, and interstitial substances all play a vital part in the regulation of wound healing processes [40], [41].

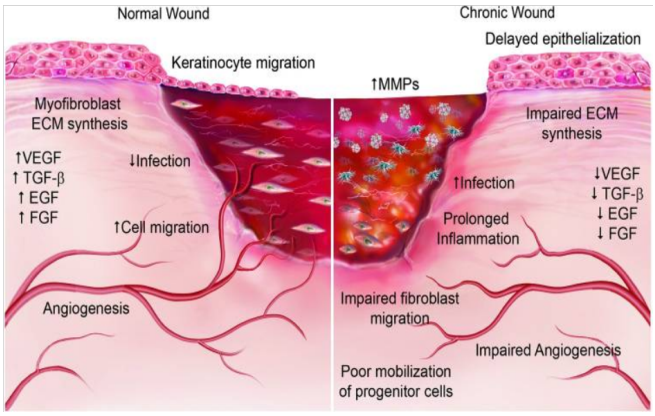

Fig. 1. Normal wound vs chronic wound. [42].

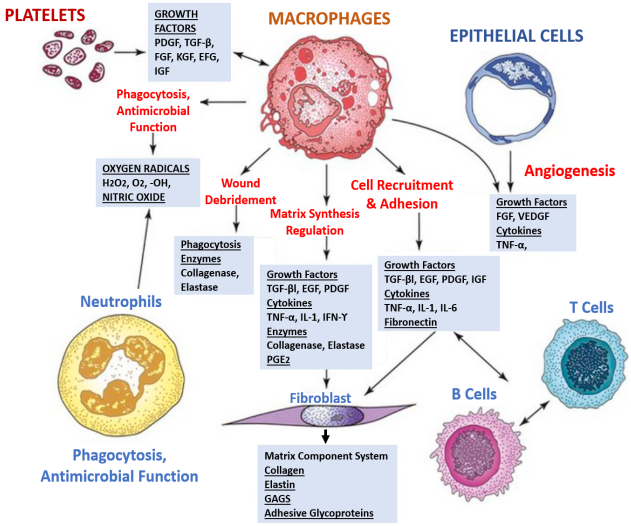

Fig. 2. Interaction & participation of different factors in wound healing [109].

B. Pathologic Wound Healing

Certain patho-physiologic or metabolic states can put their influence on standard wound healing means, due to which normal healing may be delayed, resulting in chronic wounds (wound stays for more than 6 weeks) [43], [44].

Fibroproliferative condition can develop regardless of how pathological wound healing begins. These conditions could be categorized as hypertrophic scarring and keloid growth, which are considered as over-healing reaction [45]. Longer than usual inflammation phase is associated with hypertrophic scarring, and long-standing ulcers could be linked with enhanced proteolysis, combined with diminished cellular proliferation and relocation [25], [46].

The essential growth factors which exhibit their vital role in wound healing and tissue regeneration include Platelet derived growth factor, Epidermal growth factor, Fibroblast growth factor, Insulin-like growth factor, Vascular endothelial growth factor, Transforming growth factor-beta, Hepatocyte growth factor and keratinocyte growth factor [18].

The succeeding sections go over above growth factors' roles in the wound repair procedure.

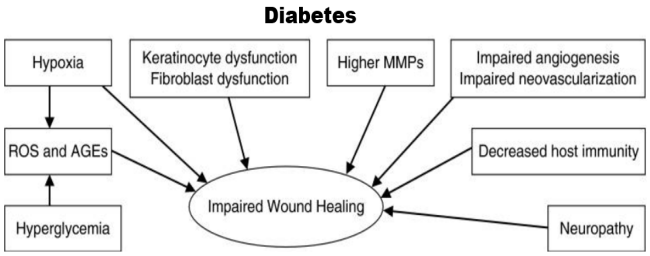

Fig. 3. Causes of impaired wound healing in diabetes [110].

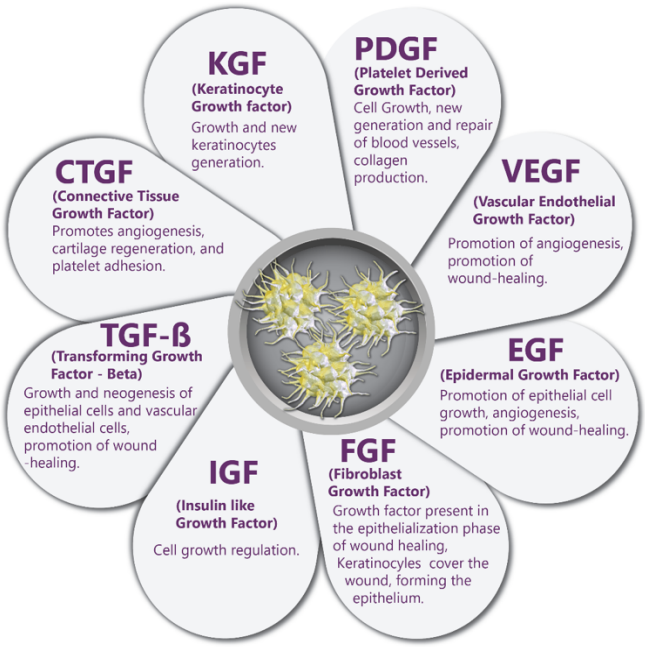

Fig. 4. Platelet growth factors, enveloped in Alpha granule.

C. Platelet-derived Growth Factor (PDGF) – Importance in Tissue Repair and Regeneration

PDGF is known as initial molecules excreted when a lesion occurs, and it supports cellular responses in all the way through the healing process [53]. In fibroblasts and smooth muscle cells, PDGF excites a variety of reactions, including protein and collagen production, collagenase action, and migration of cells [54]. Moreover, PDGF also facilitates endothelial cell production and relocation, resulting in new vascularization [55]. PDGF also promotes TGF-beta production, which ultimately starts collagen formation [56]. PDGF has also been documented to have promising role in Insulin like growth factor-1 synthesis [54].

PDGF exhibit a powerful cellular migration influence for neutrophils and monocytes enhancing multiplication of fibroblast, vascular cells, and smooth musculature tissues, thus excites new vascularization, granulation tissues synthesis and injured tissues restoration [54].

PDGF application has been shown to be effective in treating diabetic foot ulcers [57]. The levels of PDGF in diabetics have been found to be decreased, resulting slow healing response [58], [59].

D. EGF (Epidermal Growth Factors) Is a Protein that Helps the Skin Growth

Epidermal growth factor is particularly excreted through platelets, though macrophages, fibroblasts, and MSCs may generate EGF too, and its greater concentration can be observed in the initial phase of healing [56].

EGF efficiently stimulates the production, differentiation, growth, and relocation of epithelial and keratinocyte cells. EGF exhibits a promising role in wound healing and tissue regeneration due to its ability to stimulate new blood vascularization [60], [61]. EGF has been found to help new epithelium synthesis in partial thickness burns and reduces scarring in full thickness lesions [46], [62].

E. Expansion of Fibroblast Factors (FGF)

FGF promotes epithelial regeneration, new vessels formation, and the development of granulation tissue [63].

It stimulates epithelialization indirectly by boosting Transforming growth factor-alpha [64]. Along with VEGF, FGF is present throughout the proliferation phase. FGF promotes fibroblast production, collagen synthesis, and speed up the formation of granulation tissue [46]. Animal model research has yielded positive results, such as quicker lesion repair in a rat pattern [65]. FGF is shown to have a beneficial impact on hypertrophic scar control causing scarring reduction [66].

F. Insulin Like Growth Factors (IGF)

It has been established that the Insulin like growth factor promisingly participates in proliferative and inflammatory stages [57]. Insulin like growth factor-1 (IGF-1), which is generated by fibroblasts, has autocrine impact on themselves [53]. It has been demonstrated that IGF levels are low in chronic diabetic wounds. Application of IGF-1 exogenously demonstrates enhance wound healing in diabetic rats, normal rats, and rabbits [67]-[70]. It has been shown that when different growth proteins, consisting of platelet-derived growth factor and epidermal growth factor, are used in conjunction with IGF, it has a more substantial effect on keratinocyte migration and tissue healing [71], [72].

G. Vascular Endothelial Growth Factors (VEGF)

A significant paracrine impact of VEGF on endothelial cells during wound healing is encouraging and boosting wound angiogenesis, as a result, it is the primary growth factor in granulation tissue, where it begins the angiogenesis process [58], [73]. Several growth factors belonging to the VEGF family have been demonstrated to have significant roles in new vessels development, lymphatic vessels development, and vessels permeability [74]. By increasing epithelialization, new blood vessels development, and granulation tissue formation, VEGF therapies have been shown to accelerate wound healing successfully in diabetic wounds and ischemic ulcers [75]-[77].

H. Transforming Growth Factor-beta (TGF-β)

TGF-beta is divided into 3 isoforms: TGF-beta1, TGF-beta2, and TGF-beta3, each of which has significant wound-healing characteristics [25]. The TGF-beta1 is more abundant during the wound healing phases [44].

TGF-beta3 is linked with new vessels formation, while TGF-beta1&TGF-beta2 are linked with fibroblast stimulation and diversification of extra-cellular substances buildup, lesion contraction or scar production [78]. TGF-beta stimulate macrophages to migrate, also initiate the formation of collagen and fibronectin while hindering the action of metalloproteinases [46]. The importance of TGF-beta3 is to minimize collagen deposition throughout the proliferation and re-modeling phases, resulting diminishing scar production [79]. Furthermore, TGF-beta1 deficiency has also been linked to hypertrophic scarring and keloid development in fibroproliferative ailment [78]. TGF-beta3 exerts paracrine actions on keratinocytes resulting in re-epithelialization of excisional wounds [80].

I. Hepatocytes Growth Factor (HGF)

HGF is excreted through mesenchymal cells, which is responsible for cellular growth control, and has influential role in dermal repair, granulation tissue production and new vessels formation [81]. It has also been established that the combination of HGF and VEGF has a synergistic impact on endothelial cells, which increases the angiogenic activity at the level of the wound [82].

J. Keratinocyte Growth Factors (KGF) Is a Protein that Helps to Stimulate the Proliferation of Keratinocytes

KGF stimulates keratinocyte production and relocation throughout the wound healing process, with the most significant effects during the re-modeling stage. It is demonstrated that KGF-2 stimulates the production of epithelial cells while also speeding the healing of venous ulcers [46].

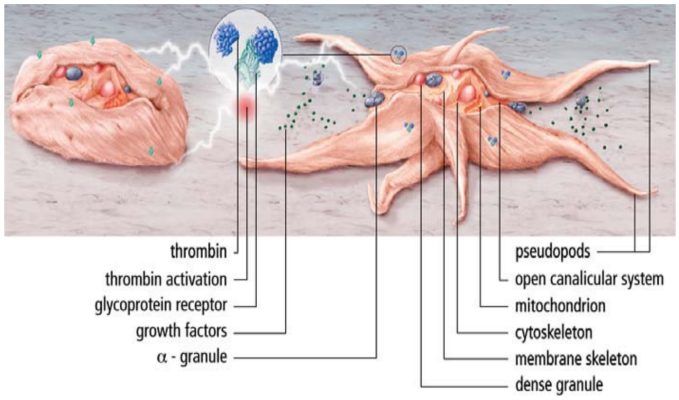

Fig. 5. Resting vs activated platelets [111].

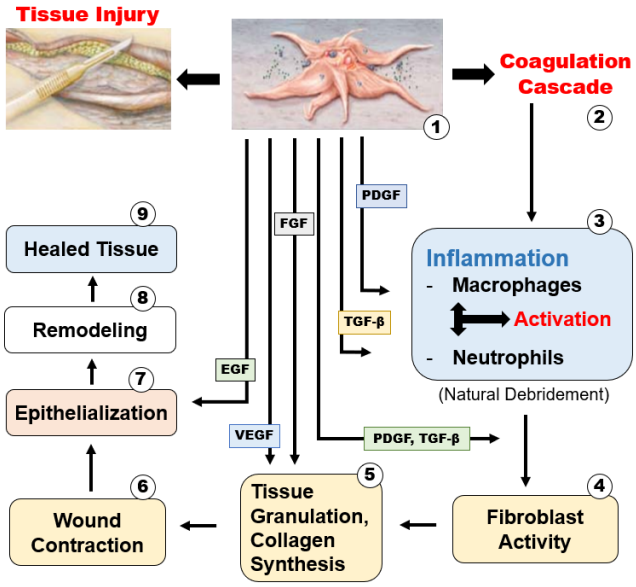

Fig. 6. Platelet derived growth factors' role in different phases of wound healing [111].

K. PRP Preparation Techniques, as well as Therapeutic Formulation

Platelet-rich plasma is known to have a higher concentration of platelets than whole blood, which results in a higher concentration of growth factors to have Growth factors rich plasma [83]. Currently, PRP is being utilized extensively in musculo-skeletal injuries [84], soft tissue injuries [85], nerve tissues [86], long standing cutaneous wounds/ulcers [87], certain eyes related conditions/problem [88], and dental procedures [89]. Platelet-rich plasma is classified by FDA as least engineered autologous blood substance [90]. Another significance of PRP among many is that it is simply extracted from person's own blood with a certain time-period of centrifugation, therefor it is considered as secure, straightforward, and economical product [91], [92]. Through regulating the centrifugation factors and PRP activation method, the contents of growth factors and protein released from platelets can be monitored [93].

It is considered that there is no clear standardized protocol for platelet-rich plasma preparation at this time, and it is still unregulated and unstandardized [91]. Conditions for centrifugation are essential to have high quality of platelet-rich plasma with the collection and identification of blood platelets [91]-[94].

The terminology and meaning of PRP have recently been subjected to intense debate [95],[96]. PRP preparation must be step-by-step and detailed with information of anticoagulant usage, centrifugation criteria and its speed, RBCs or WBCs presence within PRP and platelet activation protocol [90],[96]. Studies suggest that WBCs and RBCs should not be present in the final product of Platelet-rich plasma due to their unwanted effects on growth factors (WBCs) and oxygen free radicals' formation that can disrupt the wound [97].

In this study, Growth factors-rich plasma system named "Prizmah" was used. "Prizmah" is a brand of AK Pharma, Inc., USA. The Prizmah system has the outstanding ability to have autologous platelet substance in 4 forms, named Platelet-rich plasma (liquid), Growth factors-rich plasma (liquid), Growth factors-rich plasma (Gel) and Growth factors-rich membrane. To have the Growth factors-rich membrane, specialized Prizmah tray is used.

L. Method of Preparation – Prizmah System (AK Pharma, Inc., USA)

Through the Prizmah system, for each patient treatment, 18ml blood is collected into two Prizmah sterilized blood-collecting tubes (9ml in each tube) having sodium citrate functions as an anticoagulant and a gel separator. These tubes are centrifuged at the speed of 3000 rpm for 7 minutes. After centrifugation, PRP is obtained without RBCs and with around 10% of WBCs fraction. This PRP is then transferred into the Prizmah activation tube (having a certain amount of calcium chloride) through completely closed system transfer equipment. Calcium provides stimulus for platelets' activation, and Alpha granules of platelets start ejection of growth factors into the plasma. The plasma in the activation tube can now be named Growth factors-rich plasma. This substance is now ready to inject at the corners of the wound. If Growth factors-rich plasma remains in the activation tubes for 20-25 minutes, it becomes gel due to fibrin thread formation. Now it is classified as Growth factors-rich plasma gel. For membrane formation, the gel is placed on the surface of the Prizmah tray, which is then covered with a tray lid. Due to the pressure exerted by the tray lid, within 2-3 minutes, plasma present in the gel flows down into the tray chamber, and a white-colored Growth factors-rich membrane is obtained under the tray's lid. This membrane is the collection of growth factors and fibrin thread and is placed over the wound for speedy healing. The plasma that comes out from the Growth factors-rich gel and is present in the bottom chamber of the Prizmah tray is collected in a syringe and is used to wash the wound. Thus, through the Prizmah system, Growth factors-rich plasm (liquid) can also be injected at the corners of the wound, and it is also possible to have a Growth factors-rich membrane to put over the wound.

A complete photographic protocol of Prizmah system is shown in Fig. 7.

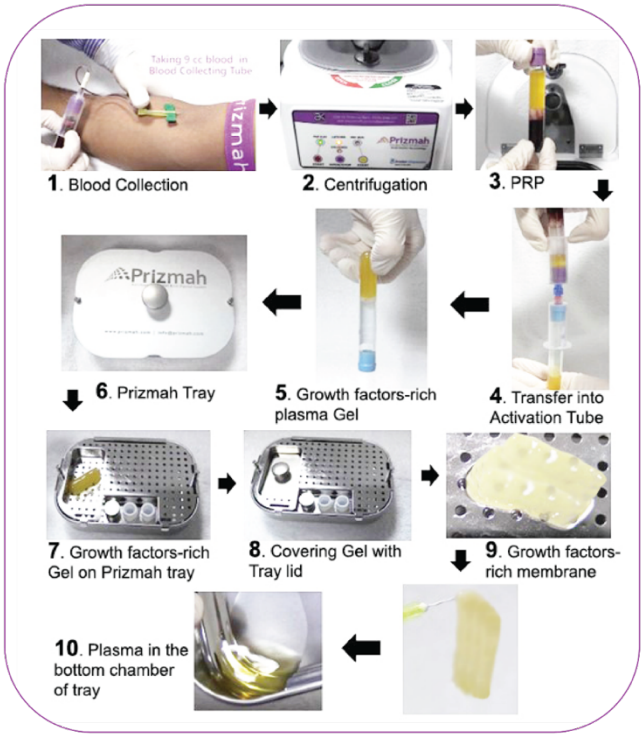

Fig. 7. The Prizmah system: growth factors-rich plasma, gel & membrane.

II. Material and Method

A. Patients

Three patients were selected with type 2 diabetes between the ages of 50 and 55 with a three-month history of nonhealing ulcer on lower limb. Patients do not have any cardiovascular symptoms/disease or other comorbidities.

The protocol included the debridement of the wound, strict diabetic control, proper offloading of the wound and use of appropriate antibiotic if needed. After debridement the ulcer must not have necrotic fragments, foreign particles, sinus tracts, tunneling, and undermining. The wound exhibits uncompromised vascular tissue after debridement. Moreover, the limb possesses adequate perfusion as revealed through

examination and non-invasive vascular testing ankle brachial index (ABI) and toe brachial index (TBI).

B. Protocol

Growth factor-rich plasma, gel and membrane is prepared from the Prizmah system. After debridement, the wound was washed properly with an antiseptic solution and then with plasma obtained after Growth factor-rich membrane formation. Growth factors-rich plasma is then injected (0.5 to 0.8 ml) around the corners of the wound (1 to 1.5 cm apart) up to the level of hypodermis. After the application of growth factors-rich plasma, growth factors-rich membrane is placed over the wound and dressed. The protocol is repeated every week with proper debridement of the wound. The promising results were noticed within 3rd application (Fig. 8, 9 and 10).

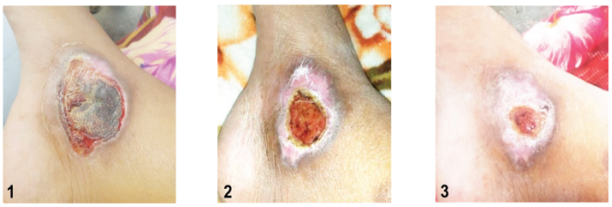

Fig. 8. 1) Before Growth factors-rich plasma/membrane application.
2) After 3rd Application.
3) After 5th Application.

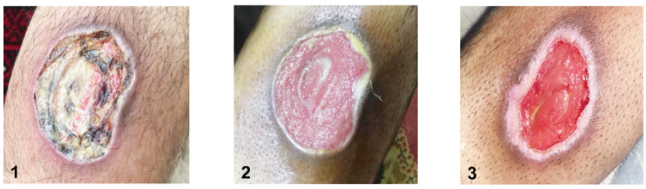

Fig. 9. 1) Before Growth factors-rich plasma/membrane application.
2) After 3rd Application.
3) After 6th Application

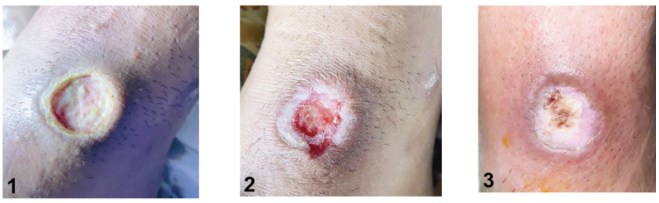

Fig. 10. 1) Before Growth factors-rich plasma/membrane application.
2) After 3rd Application.
3) After 5th Application

III. Application of Growth Factors-Rich Plasma in the Healing of Cutaneous Wounds

The use of new cellular treatments, such as platelet rich-plasma/growth factors-rich plasma, has risen in recent decades due to their potential use as therapeutic agents in a wide range of regenerative treatments [98]. The medicine industry is striving to develop therapies that are less intrusive and more economical in order to improve final results [94]. PRP application provides cytokines and growth factors to the injured tissues, controlling inflammation, new blood vessels formation, and tissue regeneration [34], [94]. PRP application is effective in treating a variety of injuries. To keep tissues in a state of homeostasis, the body regulates the processes of angiogenesis, controlling inflammation, restoration, and regeneration [99]. Many advantages exist in employing platelet-rich plasma for dermal wound healing and tissue regeneration, include the convenience of administration, low cost, longer duration of action than conventional products, and patient safety because of autologous substance [100], [101]. Platelets' hemostatic properties, as well as their high quantities of growth proteins and cytokines, aid in the healing of wounds [102].

It is common practice to utilize platelet rich-plasma/growth factors-rich plasma for the treatment of patients with chronic wounds such as diabetic ulcers when treatment is impeded by an inequity of pro-and anti-inflammatory substances, insufficient growth molecules concentrations, and presence of free radicals [103]. Growth proteins and related molecules are essential in controlling oxidative destruction [103]. Hypodermal growth factors-rich plasma injection and topical application of Growth factors-rich membrane or gel is used to treat non-healing lesions of various origins. Study demonstrates that patients with Growth factor-rich injections, gel or membrane application exhibited a significant reduction in damage without experiencing any adverse effects, as well as a decrease in discomfort and inflammation [104]. Same promising results were visible after topical administration of platelet-rich plasma/ Growth factor-rich plasma at the secondary lesions of necrotizing infections [105]. Another study demonstrated that topical platelet-rich plasma could also reduce injured skin further damage [106]. Further studies concluded with a prominent initiation of the relocation capability and production of mesenchymal stem cells and fibroblasts at the level of the wound. Moreover, speedy healing and new blood vessels formation with Growth factors-rich plasma is observed. Another study claimed that PRP/ Growth factors-rich plasma exerted antimicrobial action against certain skin specific bacteria as well [91], [107].

Researchers observed that the use of Growth factors-rich plasma decreased erythema and hastened recovery in an experiment that included ablative fractional carbon dioxide laser treatments [106]. Clinical investigations have shown that platelet-rich plasma/ Growth factors-rich plasma can improve facial skin stiffness, and wrinkle condition [108]. Furthermore, Growth factors-rich plasma has been proven to have an effect on scalp hair loss (androgenetic alopecia) [97].

IV. A Way Forward for Repairing the Wound

Because of recent advancements in regenerative medicine, we now have a better understanding of how wounds heal, mechanism of angiogenesis and tissue regeneration. However, the preparation of Platelet-rich plasma and Growth factors-rich plasma must be meticulously planned to ensure high-quality end product. Just platelets should be extracted within plasma among other blood components, and subsequently, platelet-rich plasma should be produced using centrifugation and ultimately Growth factors-rich plasma must be developed through proper activation methods. Studies on the long-term consequences of PRP/Growth factors-rich plasma-based skin healing are rare. Hence, large-scale controlled trials are necessary to evaluate further the effectiveness of platelet-rich plasma/Growth factors-rich plasma wound treatment.

The capacity of stem cells to repair and replenish tissues after injury makes them a promising treatment option for both acute and chronic injuries. It is well known that stem cells play an essential role in wound healing, particularly in cutaneous wounds. Their capacity to affect a lesion's capability to heal ahead of the inflammatory stage and prevent relapsing into a long-standing damage condition has been demonstrated.

V. Conclusion

Regenerative treatments are considered as novel treatment options for wound healing and speedy recovery with minimal or no adverse events. As a result of the outcomes of this study, Growth factors-rich plasm, gel and membrane have promising results in diabetic ulcers and chronic wounds. This treatment, with its inspirational outcomes proves to be a safer and economical therapy alternative for skin wound healing in the future. Its remarkable effects reduce the period of recovery and enhance the quality of life of the patients.

Disclosure

The authors declare the following competing financial interest(s):

Dr. Kashif Ali Samin declares no conflicts of interest in developing and publishing this study.

Dr. Tariq Mehmood Dar is the Director in AK Pharma Pvt. Ltd.

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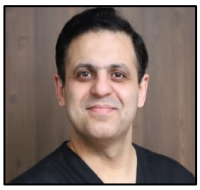

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