

Platelets Rich Plasma Accelerates Wound Healing: Histopathological Study

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ABSTRACT:

Healing of surgical or traumatic wounds in equine usually delayed and complicated. Moreover, the incidence of traumatic wounds in equine are greater than any other animals, therefore, a new therapy are required for a fast and satisfactory healing. In this study we aimed to achieve optimal healing with minimal or even no scar formation. Platelets Rich Plasma (PRP) which play an important role in wound healing due to its massive content of growth factors. In this work, we used 6 donkeys divided into 2 equal groups. In all animals, one skin wounds were created bilaterally on the back region (3×3 c, full-skin thickness). The first group received one PRP injection in the right wound directly after wounding, and saline treatment to the left wound daily. The second group received tow PRP injection in the right wound, the first directly after wounding and the second at the 14th day after wounding, while the wound of left side subjected to betadine treatment daily. Clinically, there was no great difference between single or double shout of PRP injection. The contraction occurs at 14th day, but increased after the second shout of PRP injection, while it was delayed in both saline and betadine treated groups. PRP treated groups showed complete wound closure with healthy granulation tissue, unlike the other groups. Microscopically: PRP treated wounds revealed complete epidermal and dermal formation with skin appendages. Only the periphery of wounds covered with epidermis while the center still denuded in both saline and betadine treated groups.

keywords: Skin, Wound healing, PRP, Equine.

INTRODUCTION:

One of the primary functions of wound healing is to restore the protective epithelial barrier. Without this defense, our initial protection against infection is gone, that leaves us vulnerable to outside pathogens and fluid loss. (Hailey *et al.*, 2018). Wound healing process after any intervention is considering a challenge for the surgeons, in spite of the advances in wound closure techniques and devices (Desai, *et al.*, 2013).

It is well known that the incidence of traumatic wounds in equine is higher than other species and healing usually delayed and complicated (Mickelson, *et al.*, 2016). Due to skin loss and inability to primary wound closure, the treatment of traumatic skin wounds to heal by second intention is the only available option (Lindsay, 1990; Auer and Stick, 1999). So, there is a crucial need for newer materials of enhancing the healing process to achieve optimal outcomes. Platelet-rich plasma (PRP) is an endogenous therapeutic technology which gains interest in

regenerative medicine because its ability to stimulate and accelerate tissue healing (Anitua, *et al.*, 2012). PRP is an autologous biological product derived from the patient's blood, by centrifugation process a plasma fraction is obtained with a platelet concentration higher than that present in circulating blood (Ahmad *et al.*, 2012). The use of PRP in both humans and animals increases steadily, and its healing properties in cutaneous wounds have been reported in many clinical and experimental studies with dogs (Jee *et al.*, 2016), horses (Carter, *et al.*, 2003b), humans (Suthar, *et al.*, 2017), and other species (Lee *et al.*, 2008; Kimura *et al.*, 2005).

PRP also used as clinical tool for several types of medical treatments, including tendinitis (Mishra, *et al.*, 2009), osteoarthritis (Andia & Abate, 2013), nerve injury (Yu, *et al.*, 2011), cardiac muscle injury, bone repair and regeneration (Griffin, *et al.*, 2009).

Platelets play an important role in the wound healing process due to hemostatic functions and concentrated levels of growth factors and cytokines (Brissett & Hom, 2003). A higher concentration of growth factors helps in the regeneration of epithelial and endothelial cells, and also stimulates angiogenesis, collagen deposition, and accelerates the healing process (Jee *et al.*, 2016). PRP showing a bio regenerative action, by stimulating fibroblast proliferation, increasing anti-inflammatory factors, angiogenic factors and proteins which are related to extracellular matrix remodeling (Kim *et al.*, 2011).

Degranulation of platelets causes them to release these growth factors which include transforming growth factor- β (TGF- β), fibrinogen, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-A (TGF- α), vascular endothelial growth factor (VEGF), platelet thromboplastin, thrombospondin, coagulation factors, calcium, serotonin, histamine, and hydrolytic enzymes (Vendramin, *et al.*, 2006).

TGF- β is particularly important because once it is activated it affects most aspects of tissue repair, including initiation and termination in the

treatment of chronic, non-healed or slow to heal wounds (Carter *et al.*, 2003b).

The PRP gel is a product that accelerates the healing of difficult-to-heal wounds. The initial phase of the healing is mediated by growth factors secreted by the platelets. The platelets were activated with thrombin, a naturally occurring platelet activator that promotes wound healing. So it induces a reduction in wound size, increases the fibroblast to macrophage ratio and increases proliferating fibroblast (Strukova *et al.*, 2001).

The current study was conducted to evaluate the effectiveness of platelet-rich plasma (PRP) on the healing of a full-thickness skin defects in equine.

MATERIALS AND METHODS:

1- preparation of platelet Rich Plasma (PRP)

A blood sample was drawn while using Acid Citrate Dextrose A (ACD-A) as anticoagulant. Then, Gradient density centrifugation was made to obtain the platelet rich plasma layer according to the method described in (Andia *et al.*, 2012). The volume of PRP needed depends on the wound surface area. In general, the wound surface should be completely covered with the gel. Five to ten ml of PRP is sufficient for most wounds (Everts *et al.*, 2006).

2- Experimental design

The current study followed the animal welfare guidelines of the faculty of veterinary medicine, university of Sadat city, Egypt.

Animal grouping

Six adult Egyptian donkeys (with body weight ranged from 200 to 250 kg) with normal physical examination except they suffered from fractures in one of their limbs and take a decision of execution from the veterinarian, were used in the current study. Animals were housed in covered stalls, they were allowed free choice grass hay all over the day and a one kilogram of concentrates at night, and free-choice water throughout the study.

Animals were divided into 2 equal groups. In all animals, one skin wounds were created bilaterally on the back region (3×3 c, full-skin thickness). The first group received one PRP injection in the right wound directly after wounding, and saline treatment to the left wound daily. The second group received tow PRP injection in the right wound, the first directly after wounding and the second at the 14th day after wounding, while the wound of left side subjected to betadine treatment daily.

3- Surgical procedures

On the day of surgery, tetanus prophylaxis and flunixin meglumine (50 mg/ 45 kg, IV, Schering-Plough, Germany), were administered to the animals. Skin of the back region was clipped and aseptically prepared with povidone-iodine. Animals were anesthetized using xylazine hydrochloride (2% xylazine hydrochloride "xylaject"; ADWIA, 10th of Ramadan City, Egypt) at a dose of 1.1 mg/kg, IV, and Lidocaine HCL (2% Lidocaine HCL "lidocarp"; ALDEPEIKY (DPK), ELobour City, Egypt) at dose of 1ml/cm²

4- Assessment of wound healing

Macroscopic evaluation

Digital photographs were taken of all wounds after the area had been carefully cleaned using saline to visualize wound margins. The width and the length of wounds were measured using digital caliber.

The surface area was estimated using the following formula:

$$\text{Wound surface area} = \text{Length (mm)} \times \text{Width (mm)} = \dots \text{ mm}^2$$

The rate of wound contraction was evaluated by determination of the unclosed area of the wound / time. It was calculated using the following formula:

$$\text{Wound surface area percent relative to day zero (WSAP)} = (\text{wound surface area} \times 100) / (\text{surface area of the excision at day zero})$$

$$\text{Wound contraction percent (WCP)} = 100\% - \text{WSAP}\% = \dots\% \text{ (Zaid et al. 2017).}$$

Histopathological examination:

Animals were euthanized under the effect of thiopental sodium anesthesia (Thiopental®: EPICO Co., A.R.E). Skin samples were obtained from the whole wound tissues (healed or not) and the surrounding intact skin. Samples were fixed in 10% neutral buffered formalin for 72 hours and then trimmed and processed for Haematoxylin and Eosin and in order to evaluate the structural organization of collagen in the dermis of the repaired wound, a Masson's trichrome stain was performed. Histological photos were taken by using Leica EC3 digital camera.

RESULTS

Physical evaluation of the wounds:

The tested compounds in this study did not cause any side effects throughout the experiment. The square shape of the wound was remained till the end of the experiment.

Wound expansion was observed till the contraction started at the day 14th. PRP treated wounds contract firstly and increased steadily and evenly especially after the second shot of injection. The contraction in both control groups (saline, betadine) started at day 18th, and it was uneven and delayed.

In saline treated group, wounds revealed delayed closure of wound edges, delayed crustation and formation of unhealthy granulation tissue with uneven contraction of wound edges. Blood tinged secretion was oozed from the wounds and hyperemia was observed for about 10 days. In this group wounds were still unclosed at the final day of the experiment (**fig.1**).

The gross evaluation of the betadine treated group revealed incomplete closure of the wounds at the final day of the experiment with unhealthy granulation tissue (**fig.2**).

At PRP treated wounds, both one shot injection & double shot injection the wounds showed complete epithelization, healthy granulation tissue formation and closing of the surface (**fig.3,4**).

Histopathological examination

Histopathological examination of induced wounds treated with saline as a negative control showed epidermal regeneration at the margin of the wound, incomplete epithelization and the under-granulation tissue also showed formation of melanocytes in stratum basalis and stratum corneum at the epidermal surface. Masson's Trichrome stain Showed formation of collagen and elastic fibers in the wound surface.

Betadine treated group (C+ve), showed the center of the wound which showing epidermal regeneration at the margin of the wound only, incomplete epithelization and also show the under-granulation tissue, weak keratin layer formation was observed at the epidermal surface. Masson's Trichrome stain Showed, formation of collagen and elastic fibers within the granulation tissue under the epidermis at the margin of the wound.

While the one shout PRP treated group showed, epidermal regeneration at the entire surface of the wound which completely covering the under-granulation tissue, keratin layer formation was observed, also there were epidermal projection in the under-granulation tissue and sweat gland formation. Masson's Trichrome stain Showed, keratin layer on the top of the epidermis and granulation tissue formation.

Double shout PRP treated group showed, formation of normal epidermi, hair corpuscle (HC), sebaceous gland (SG) and subepidermal smooth muscle. formation of melanocytes in stratum basalis and stratum corneum at the epidermal surface were observed. Masson's Trichrome stain Showed, complete epidermal layer covering the entire wound, sebaceous glands and sweet glans in the under-granulation tissue.

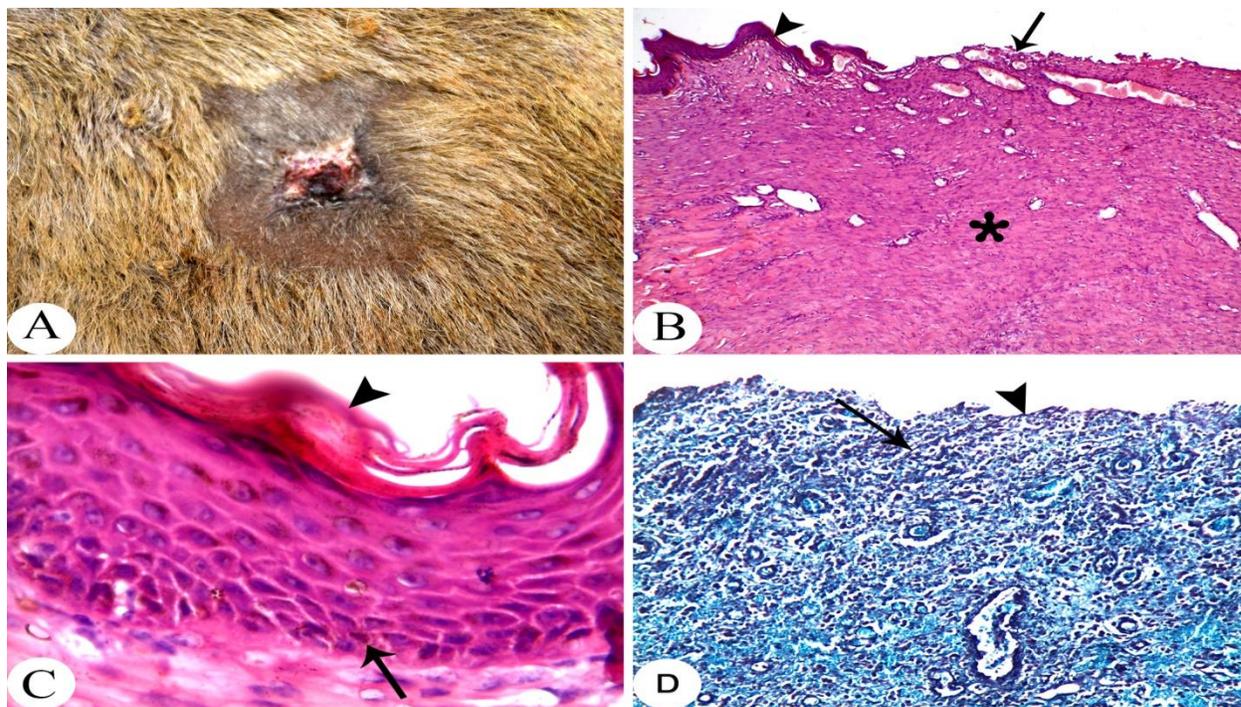


Fig. (1). Saline treated wound. **A)** showing the gross picture of the wound which not completely healed. **B)** Microscopic picture of the center of the wound which showing epidermal regeneration at the margin of the wound (arrowhead), incomplete epithelization (arrow) and the under-granulation tissue (asterisk). X 4. **C)** High magnification from Fig. B showing formation of melanocytes in stratum basalis (arrow) and stratum corneum at the epidermal surface (arrowhead). X 40. **D)** Showing formation of collagen and elastic fibers in the wound surface (arrow). Trichrome stain. X 10.

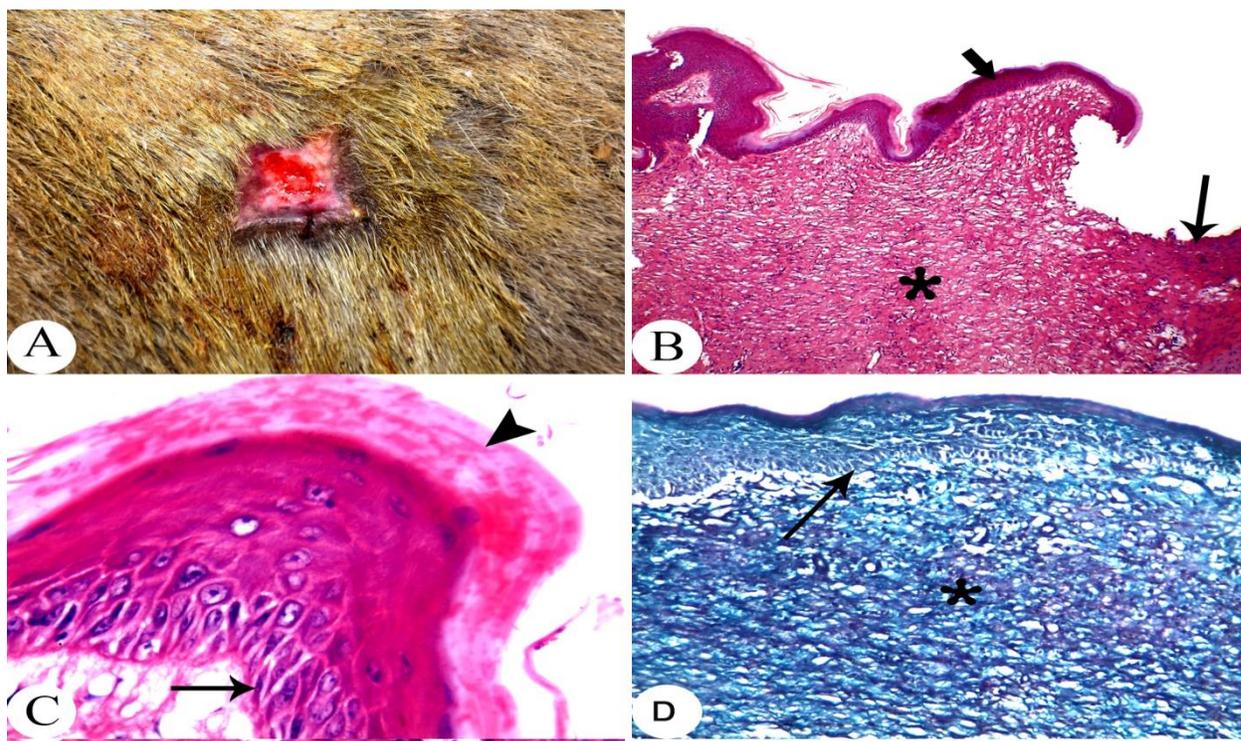


Fig.(2). Donkey Skin. Betadine treated wound. A) showing the gross picture of the wound which not completely healed. B) Microscopic picture of the center of the wound which showing epidermal regeneration at the margin of the wound (thick arrow), incomplete epithelization (thin arrow) and the under-granulation tissue (asterisk). X 4. C) High magnification from Fig. B from showing weak keratin formation at the epidermal surface (arrowhead), and incomplete epidermal formation (arrow). X 40. D) Showing formation of collagen and elastic fibers within the granulation tissue (asterisk) under the epidermis (arrow) at the margin of the wound. Trichrome stain. X 10.

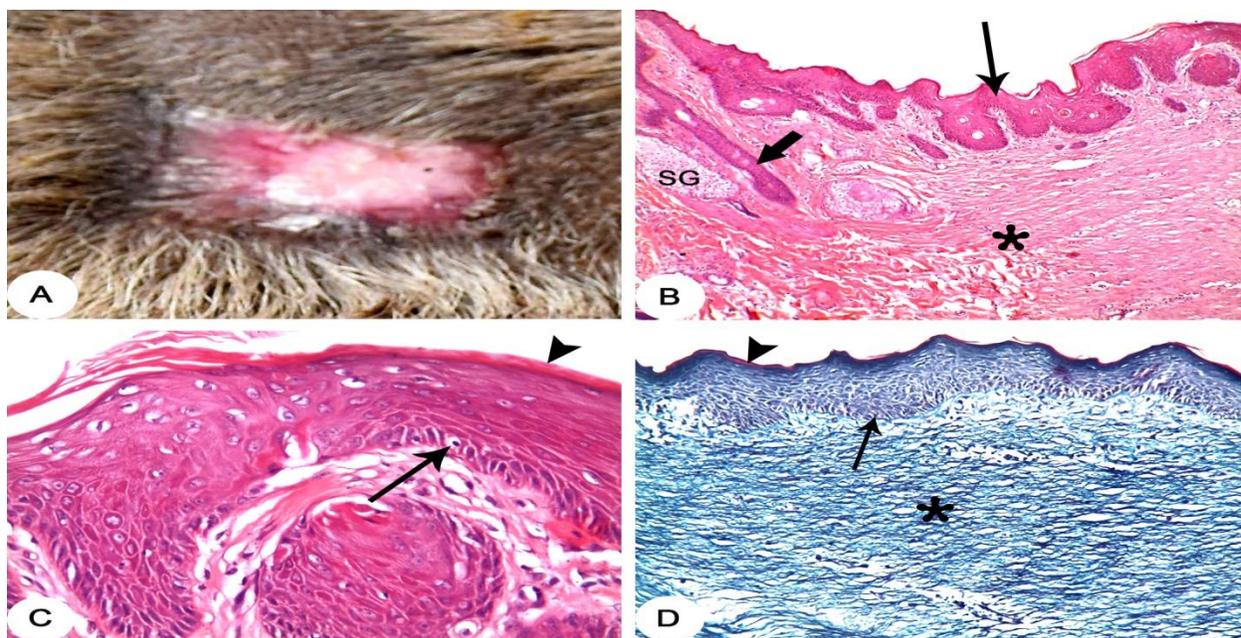


Fig. (3). Donkey Skin, PRP treated group, one shot. A) showing the gross picture of the wound which healed with complete epithelization. A) Showing epidermal regeneration at the entire surface of the wound (arrow) which completely covering the under-granulation tissue (asterisk). HE stain. X 4. B) Showing keratin layer formation (arrowhead), epidermal projection (thick arrow) in the under-granulation tissue (asterisk) and sweat gland formation (thin arrow). HE stain. X 10. C) Showing keratin layer (arrowhead) on the top of the epidermis (arrow), and granulation tissue formation (asterisk). Trichrome stain. X 10. D) Showing formation of collagen and elastic fibers within the granulation tissue (asterisk) under the epidermis (arrow) at the margin of the wound. Trichrome stain. X 10.

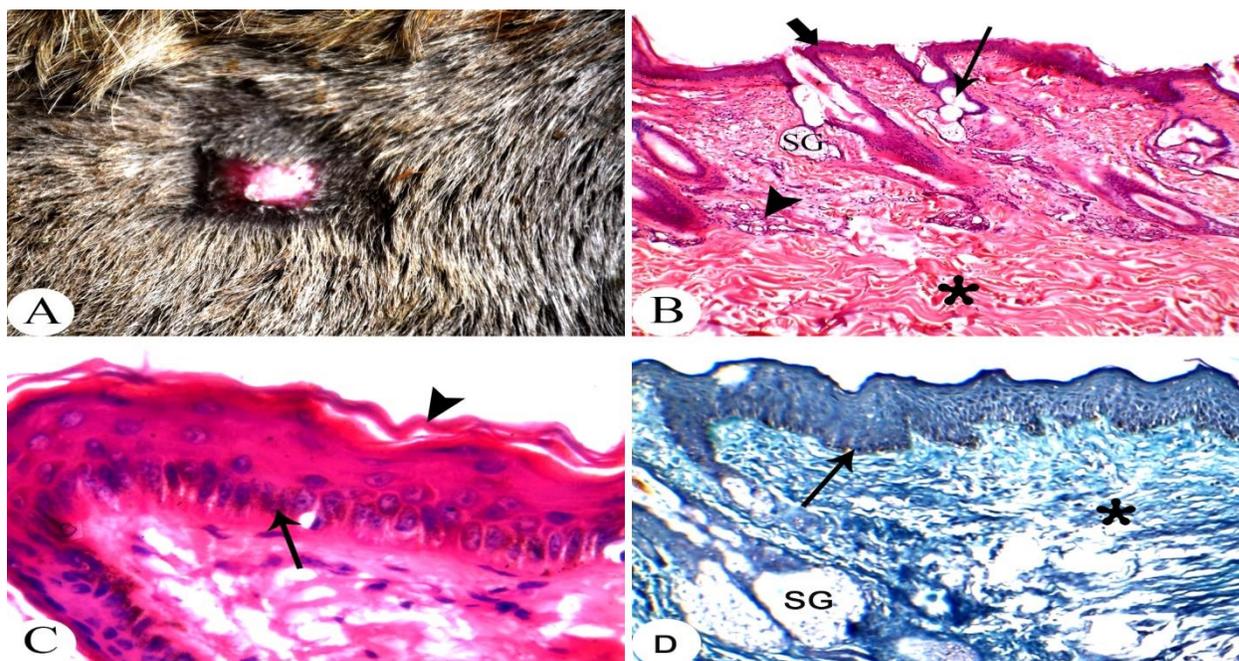


Fig. (4). Donkey Skin, PRP treated group, double shot. A) showing the gross picture of the wound which healed with complete epithelization. B) showing formation of normal epidermis (thick arrow), hair corpuscle (HC, thin arrow), sebaceous gland (SG) and subepidermal smooth muscle (asterisk). X 10. C) High magnification from Fig. B showing formation of melanocytes in stratum basalis (arrow) and stratum corneum at the epidermal surface (arrowhead). X 20. D) Showing complete epidermal layer covering the entire wound (thin arrow), sebaceous glands and sweat glands in the under-granulation tissue (asterisk). Trichrome stain. X 4.

DISCUSSION:

In this work, we try to evaluate the effect of PRP injection on wound healing in equine skin and compare between single and double injection. The contraction in PRP treated group may be attributed to the activated platelets which induces a reduction in wound size by, increases the fibroblast to macrophage ratio and increases proliferating fibroblast according to (Strukova *et al.*, 2001), also may be due to releasing of VEGF (Vascular endothelial growth factor) which is a mediator of angiogenesis that stimulates endothelial cell proliferation and also promotes fibroblast proliferation as indicated by (Kliche & Waltenberger, 2001).

Growth factors also induces smooth muscle cell migration as reported by (Hosgood, 1993). Epidermal growth factor (EGF) is also released by platelets and considers as a chemotactic for fibroblast so accelerates the rate of epidermal regeneration and increases wound tensile strength as reported by (Adelmann-Grill, *et al.*, 1990). An increased number of fibroblasts in our PRP-gel wounds may help in explaining the

increased collagen and repair in the dermis following the treatment and this supported by (DeRossi *et al.*, 2009).

The saline group (control group) presented secretion and hyperemia for long time this may explained as evidencing late exit of the inflammatory phase according to (Abu-Al-Basal, 2010).

Also Assoian, *et al.*, (1983) can explain why that couldn't occur in PRP treated group as it was reported that PDGF increase the tissue vascularization which Increases the local circulation at the site of wound, promote fibroblast proliferation, increase collagen formation and stimulate the production of granulation tissue.

The healthy granulation tissue formation and the complete re-epithelialization of the wound site at PRP treated group, could be explained by the transforming growth factors (TGF: TGF- β 1 and TGF- β 2) which activate fibroblasts for formation of pre-collagen, which induces the deposition of collagen which lead to wound

healing this explained (Carter *et al.*, 2003b), also the platelets act in the hemostasis; wound healing and re epithelialization, releasing growth factors (GF) that stimulate the angiogenesis, promoting growth and vascular fibroblast proliferation which in turn provide an increase in the collagen synthesis this reported also by (Robson, 1997; Marx, 2004).

Microscopically the wounds in both control groups (saline, betadine) showed epidermal regeneration at the margin of the wound but the center of the wound still completely denuded, there was migration of the epidermal cells on the wound surface and infiltration of macrophages in the granulation tissue. Masson's Trichrome stain showed formation of collagen and elastic fibers within the granulation tissue under the epidermis at the margin of the wound.

The findings in PRP treated group at (34-day post wounding - End stage), showed epidermal regeneration at the entire surface of the wound which completely covering the under-granulation tissue, also with keratin layer formation, epidermal projection in the under-granulation tissue and some skin appendages like (sweat gland formation). All these findings referred to many factors, the activation of platelets leads to releasing of many growth factors help in tissue regeneration and repair also induce fibroblast and smooth muscle cell migration and proliferation this agreed with (DeRossi *et al.*, 2009; Vendramin *et al.*, 2006; Hosgood, 1993), these factors like, VEGF (Vascular endothelial growth factor) which is a mediator of angiogenesis that stimulates endothelial cell proliferation as mentioned by (Kliche & Waltenberger, 2001), EGF (Epidermal growth factor) is also released by platelets and considers as a chemotactic for fibroblast so it can accelerate the rate of epidermal regeneration and increases wound tensile strength at the same time, this reported by (Adelmann-Grill *et al.*, 1990), the transforming growth factors (TGF: TGF- β 1 and TGF- β 2) which play role in activation of fibroblasts leading to formation of pre-collagen, which induces the deposition of collagen and increase its formation, also increase the tissue vascularization, and stimulate the production of granulation tissue by cororally this lead to

increasing wound tensile strength. This confirmed by (Assoian *et al.*, 1983; Bennett & Schultz, 1993).

Finally, PRP injection in the skin wound, either single or double shout, accelerate wound contraction and healing. A complete epidermal and dermal layer were observed covering the whole wound surface fastest than saline and betadine treated wounds. In both single and double PRP injection methods, the wounds healed nearly at the same time, but the epidermal layer of double PRP injection wound appear better than those of the single shout.

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