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Effects of Autologous and Homologous Platelet Rich Plasma on Full Thickness Autologous Skin Graft in Dogs

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ABSTRACT

Background: Wound healing is a complicated mechanism that includes inflammatory, proliferative and remodelling stages. Platelet rich plasma (PRP) is a small volume of blood plasma fortified with platelets and growth factors that enhance the healing by stimulating angiogenesis and formation of granulation tissue.

Methods: The present study was performed to evaluate the effect of autologous and homologous platelet rich plasma on full thickness dermatological reconstructive surgery in dogs. The dogs were divided in two groups A and B, each with four clinical cases. In groups A and B, autologous and homologous PRP was used respectively as skin graft augmentation. The macroscopic parameters of the study included colour, edema, exudation and histopathological parameters included acute inflammation, fibroblast proliferation and granulation tissue formation. All these observations were made on days 3, 7 and 14 post-operatively.

Result: On the basis of findings of this study, it was concluded that autologous PRP is a better graft healing promoter in terms of self-limiting acute inflammation, fibroblast proliferation, granular tissue and early healing of graft as compared to homologous PRP.

Key words: Autologus Platelet Rich Plasma, Canine, Homologus Platelet Rich Plasma, Skin grafting.

INTRODUCTION

Healing of wound is a complicated mechanism that includes different stages controlled by different growth factors. The prime goal of wound treatment is a speedy wound closure and a functional scar formation. Platelet rich plasma (PRP) may enhance the local availability of growth factors and may hasten the healing process. PRP is a small fraction of blood plasma rich in platelets and different growth factors (Kazakos *et al*. 2009). This accelerate the tissue healing by regulating angiogenesis, chemo-taxis and cell proliferation (Anitua *et al*. 2007).

Wound healing is delayed in chronic wounds due to lack of the growth factors owing to decreased production, release, trapping, excessive degradation, or a combination of these mechanisms (Waiker and Shivalingappa, 2015) but use of autologous PRP mimics the last steps of coagulation cascade by providing these growth factors as well as aiding in achieving stable haemostasis and instant adhesion of graft to bed. Moreover, autologous property minimizes the chances of immunogenic reactions and disease transmission. Hence, it can be effectively used in orthopedic procedures, sports medicine and maxilla-facial surgeries (Anitua *et al*. 2006). Studies have shown that PRP improves healing of alveolar cleft, mandibular reconstruction and osseo-integrated implants due to its adhesive nature (Albanese *et al*. 2013), cytokines, growth factors enrichment and coagulating properties (Raghoebar *et al*. 2005).

PRP is also proclaimed to facilitate wound-healing for skin burns, plastic or cosmetic surgeries. Use of PRP in reconstructive surgeries facilitates tissue handling, adaptation, vascularisation and a fast wound healing. Use of skin grafts secures a paramount importance for the treatment of defects mostly encountered in trauma, burn and acute ablative injuries because they replace dermal collagen and also offer biological barrier to wound. Different types of grafts are in practice but mesh grafts are mostly used because of least chances of hematoma formation and a good contact with graft bed (Hermeto *et al*. 2012).

In small animal clinical practice frequency of cases involving large areas of skin damage or skin loss is very high and this is followed by several complications during the healing phase of such wounds. In such cases healing needs a support in form of graft application for safe and speedy healing but graft rejection is very common. This clinical study was designed to evaluate the effect of autologous and homologous PRP on healing of full thickness skin mesh grafts in dogs.

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MATERIALS AND METHODS

Study design

A total of eight clinical cases of adult dogs (2-3 years old) of either gender, were randomly selected for this study. These cases included dogs with deep appendicular wounds with massive skin damage, received at walk in clinic of Department of Small Animal Clinical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan. Prior to inclusion in this study written consent was acquired from the owners of each dog.

Selected dogs were evaluated for suitability for grating based surgical procedure. Dogs selected of study were admitted in kennels for post-operative management. *Ad Libitum* supply of water along with the commercial feed twice daily was provided.

Grouping of dogs

For this clinical study the selected dogs were divided into two groups i.e. A and B, 4 clinical cases in each group. In group A and B, autologous and homologous PRP, respectively, was used as skin graft healing promoter during surgery, respectively. Reconstructive surgery using autologous full thickness skin grafts was performed on all dogs of the study.

Platelet rich plasma (PRP) preparation

30 mL of blood was drawn from jugular vein of each dog and shifted to vacutainer containing citrate phosphate dextrose. Each blood sample was subjected to double centrifugation. First centrifugation was carried out at 2400 rpm for 10 minutes (Tözüm and Demiralp 2003). Centrifugation separated the whole blood into three different fractions i.e. red blood cells (inferior layer) white blood cells and platelets (buffy coat) and platelet-poor plasma (superior layer). Under aseptic conditions the first supernatant plasma fraction (50%), adjacent to the buffy coat, was obtained. This fraction was spun again for centrifugation at 3200 rpm for another 15 minutes in order to obtain two layers i.e. the upper layer was platelet poor plasma PPP and the lower layer was the PRP (25%) (AL-Bayati *et al*. 2013).

Pre-operative preparation

Only those dogs were surgically dealt which had fresh wounds whereas, those having contaminated wounds or wounds >8 hrs were dealt as open wounds until a fresh wound appearance was observed. The wound debridement was performed on daily basis initially using chlorhexidine solution followed by daily cleaning with luke warm normal saline solution and dressing of wound with simple wound dressing. The healing period varied from 3-7 days depending upon the type and severity of wound in different cases. Prior to the surgery all dogs were kept off feed for 08 hours and a complete clinical examination along with complete blood count was performed on each dog to rule out anaemia which can alter the results by directly affecting wound healing (Slatter 2003; Fossum 2019). Before the surgical procedure, cephalic vein of each dog was catheterized with 22-24 gauge canula (B. Braun, Lahore, Pakistan) (Weidema 1985; Tilley and Smith Jr. 2011) followed by shaving of surgical site (Slatter 2003).

Anaesthesia

Propofol (Inj. Propofol 10mg/mL, Abbott laboratories, Pakistan Limited) @ 6.6 mg/kg slow iv, followed by Isoflourance anesthesia (Foran 99.9 % w/v, Abbott laboratories, Pakistan Limited) for maintenance (Franco *et al*. 2009).

Surgical procedure

The most common donor site for graft harvesting is abdominal and thoracic region so the donor site was selected depending on the size, nature and the damaged area. The size of graft to be harvested was according to the recipient site (minimum 4×4 inches) measured by sterilised scale. After positioning the anaesthetized dog, the skin of the donor site was grasped, lifted, dissected and then released from underlying tissue to evaluate the suitability as a graft. The reference lines were marked on the donor area with surgical markers to indicate the margins of the potential graft and to measure the donor site with reference to the graft size. Each graft size was harvested approx. 3-5% greater than the size of recipient site. The donor and recipient sites were disinfected by 1% chlorhexidine following the debridement of the wounds with normal saline. An incision was made along the marked reference lines and the graft was undermined to the level of muscles, at the distant end of the graft avoiding any trauma to the direct cutaneous vessels (Pavletic 2018).

Platelet rich plasma (PRP) application

PRP obtained from each patient was divided into two parts. One part was administered into the graft and the remaining one part was administered subcutaneously in surrounding tissue of graft before graft placement and fixation (as discussed above).

Graft application and wound closure

The graft was accurately placed and sutured on the recipient bed applying single layer of simple interrupted non-absorbable silk sutures (Mersilk 2-0, Ethicon). The donor site was closed using sub-cuticular pattern with monofilament Polyglactin 910 absorbable suture (Vicryl 2-0, Ethicon) followed by the skin sutures (horizontal mattress) with non-absorbable silk suture material (Mersilk 2-0, Ethicon) (Fig 1,2,3).

Post-operative management

During the post-operative period, all dogs received Cephradine (Injection Velosef, Glaxosmithkline Pakistan Ltd) @ 25 mg/kg bid, IM. for 07 days and Ketoprofen (injection Profenid 2.5% w/w, Sanofi Aventis (Pakistan) Ltd @ 1mg/kg sid, IM for 03 days (Hermeto *et al*. 2012). The graft was covered with triple-antibiotic skin ointment (Ointment Mycitracin Plus, Pfizer Laboratories Ltd.) and covered with

surgical gauze, bandage and adhesive tape after the surgery. Bandage was removed every 48 hours up to 15 days followed by healing as without any bandaging. During dressing change the gauze was abundantly wetted with sterile physiological solution (Otsuka Pvt Ltd, Pakistan), so as to avoid any trauma to capillary bed due to avulsion on graft. Sutures were removed on 10th and 14th day of procedure, from donor and recipient sites, respectively. Elizabethan collar was used in all dogs post-surgical procedure till sutures removal to avoid damage of graft or suture line by the dog.

Parameters of study

Following parameters were studied to evaluate efficacy of PRP as healing promoter for full thickness skin graft in dog.

Macroscopic evaluation *i.e*.

a) Colour
b) Edema of graft
c) Exudation of graft

Microscopic evaluation *i.e*.

a) Histo-pathological study was carried out on graft tissue (collected through punch biopsy needle) for inflammation, fibroblast proliferation and granulation tissue proliferation.

Sample collection by punch biopsy

Biopsy punch (6mm) for each graft was used**.** Punch biopsy was performed from the center of the graft by uni-directional rotation**.** Biopsy punch was removed and gently tissue section was grasped with the tissue forceps and placed in a labelled sample container having 10% neutral buffered formalin.

RESULTS AND DISCUSSION

Macroscopic evaluation

Colour of graft

Among the autologous PRP treated dogs, the graft appeared black in one dog indicating the necrosis of the graft while the grafts in remaining three dogs showed pink colour and healthy appearance as an evidence of graft vitality and good healing. In homologous PRP treated dogs, skin grafts of two dogs turned black while remaining two grafts presented pink colour. The wound colour of the autologous PRP treated dogs was reflective of a better healing and graft vitality as compared to homologous PRP treated dogs (Table 1). These observations are in agreement with the findings of Hermeto *et al*. (2012). A comparison was made to evaluate the efficacy of PRP and fibrin glue in skin grafting in dogs. The observed significant differences and reported that in PRP treated group, tissue colour turned black indicating the tissue necrosis.

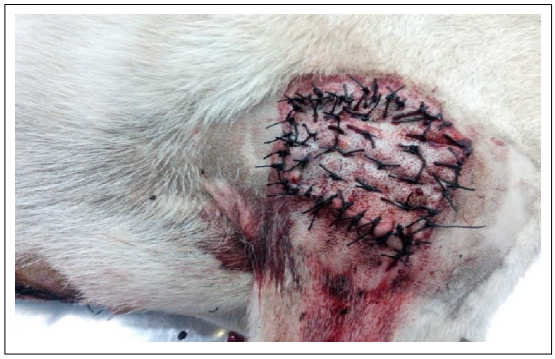

Fig 1: Skin wound before graft application.

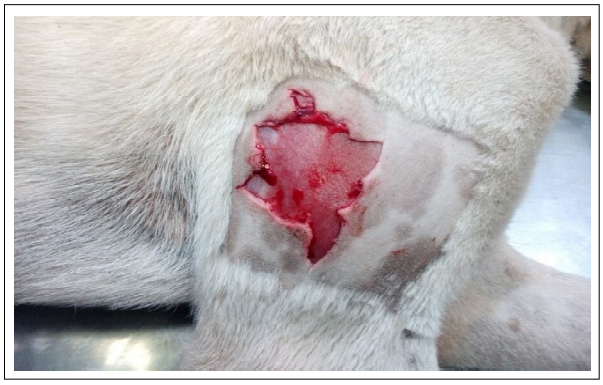

Fig 2: Wound after graft application (Autologous PRP group).

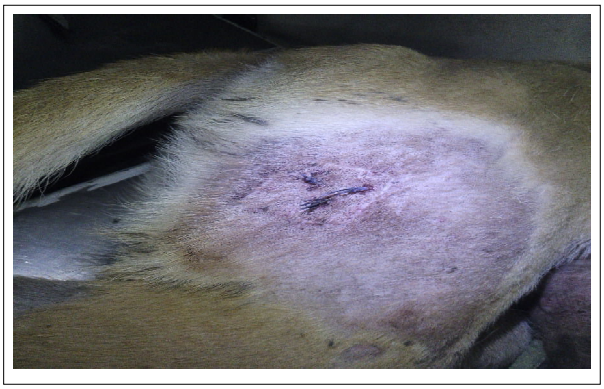

Fig 3: Day 14. Wound post graft application and sutures removal (Autologous PRP group).

Table 1: Comparison of graft colour in groups A and B.

No. of	Group A				Group B			
dogs	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
1	Pink	Yellow	Black	Black	Pink	Yellow	Black	Black
2	Pink	Pink	Yellow	Pink	Pink	Yellow	Yellow	Pink
3	Pink	Yellow	Pink	Pink	Pink	Yellow	Pink	Black
4	Pink	Pink	Pink	Pink	Pink	Pink	Yellow	Pink

Yellow: Possible infection, Black: Necrosis of graft, Pink: Normal healing.

Edema of graft

In group A, following application of autologous PRP in grafts, edema and erythema subsided rapidly reflecting a fast graft healing and no allergic reaction as compared to group B, receiving homologous PRP treatment. Among autologous treated dogs, three dogs showed mild edema and one of the dog showed moderate edema. Among homologous PRP treated dogs; two dogs showed mild edema while other two dogs showed moderate edema (Table 2). The same observations have been reported by Lee *et al*. (2008) earlier, who concluded that when a fractional resurfacing is done, surgical site edema and erythema is usually observed. No other side effects of autologous and homologous PRP application were observed in any case under this study. These findings are also in line with the observations of Bhanot and Alex (2002). The used platelet gel in human plastic surgery and documented that after platelet gel application there was significant reduction in surgical site edema as well as improvement in the rate of re-epithelialization.

Exudation of graft

All the autologous and homologous PRP treated dogs were observed for exudation at the graft site (Table 3). The exudative material was categorized as absent, serous, sero-sanguineous and purulent discharge that appeared during the healing process of graft. Among the autologous PRP treated cases, two dogs showed no exudation at all, one showed serous exudation while other one showed sero-sanguineous exudation. AL-Bayati *et al*. (2013) has reported the same observations earlier. In homologous PRP treated dogs, three dogs showed sero-sanguineous and one showed purulent exudation. The rate of re-epithelialization in skin wounds in increased with the use of PRP. Keratinocytes are differentiated into epithelial tissue with PRP application. Again the same finding are in close agreement with the findings of AL-Bayati *et al*. (2013).

Microscopic evaluation

Microscopically histo-pathological findings were recorded on days 3, 7 and 14 following graft application.

Inflammation of graft site

Acute inflammation was prominent soon after the surgical procedure in both autologous and homologous PRP treated groups however; degree of inflammation gradually reduced in autologous PRP group by day 7 while it persisted in few homologous PRP treated cases till day 14. Histo-pathological study using hematoxylin eosin staining technique revealed reduction in acute inflammation during first week after the surgery (Fig 4). The findings are in agreement with the observations of Lee *et al*. (2008). Histo-pathological examination of skin graft performed on day 3 post operative showed prominent blood vessels proliferation, inflammatory cells, fibroblasts and collagen fibers in autologous PRP treated group. This number of blood vessels, inflammatory cells and fibroblast proliferation was comparatively less in homologous PRP treated group. These observations are in line with the findings of Lee *et al*. 2008

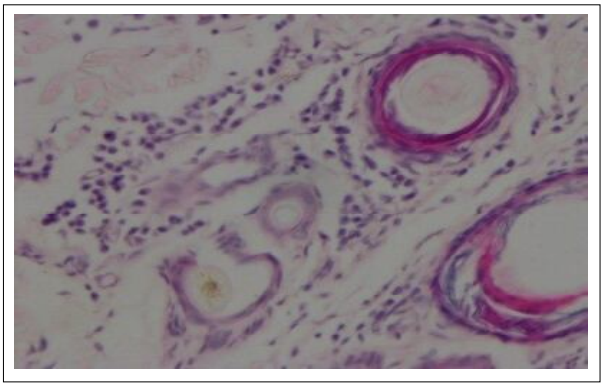

Fig 4: Inflammation of graft tissue.

Table 2: Edema of the graft site in groups A and B.

Ref No.	Group A				Group B			
of dogs	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
1	-	++	+	+	-	++	++	-
2	-	+	-	-	-	+	+	++
3	-	+	+	+	-	++	++	-
4	-	+	-	-	-	+	-	+

-: None, +: Mild, ++: Moderate, +++: Severe.

Table 3: Exudation of graft in groups A and B.

No. of	Group A				Group B			
dogs	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
1	A	A	S	SS	A	SS	P	P
2	A	S	A	S	A	SS	SS	SS
3	A	S	S	A	A	P	P	SS
4	A	SS	S	A	A	SS	S	SS

A: Absent, S: Serous, SS: Sero-sanguieous, P: Purulent.

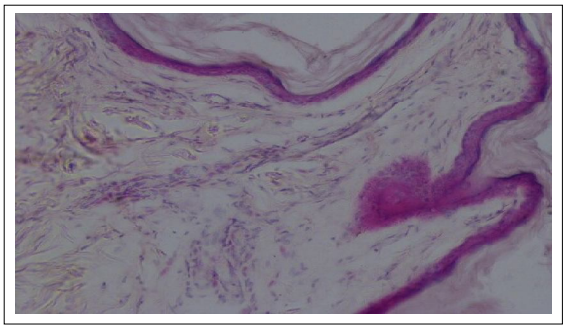

Fig 5: Fibroblast proliferation.

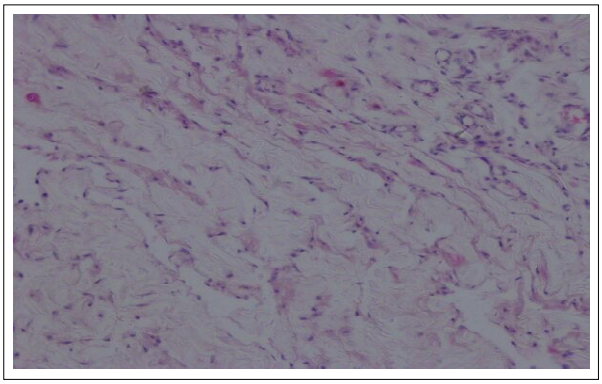

Fig 6: Granulation tissue formation.

who reported that in autologous PRP treated dogs inflammatory process subsided early as compared to homologous PRP treat group of dogs. He also concluded that epithelilzation rate was much better in autologous PRP group than in homologous PRP group. Normal structure of skin was achieved during second week because of epithelial cells, followed by quick healing of wound (Fig 7, 8).

Fibroblast proliferation

Few numbers of fibroblasts were observed in the start of the healing process in both groups but gradually; fibroblast proliferation increased in autologous treated PRP group indicating a better healing process as compared to homologous PRP treated group in which number of fibroblasts was quite less (Fig 5). These observations coincided with the study reported by DeRossi *et al*. (2009) who studied PRP in equines. Fibroblast proliferation was recorded at 3, 7 and 14 days intervals (Fig 9, 10).

Granular tissue proliferation

The histo-pathological findings on the skin biopsies harvested from different wound sites; reflected an increased wound healing followed by increased granulation tissue proliferation in autologous PRP treated group (Fig 6). The findings of this study were in line with the earlier findings of AL-Bayati *et al*. (2013). In microscopic study on day 7, granular tissues were

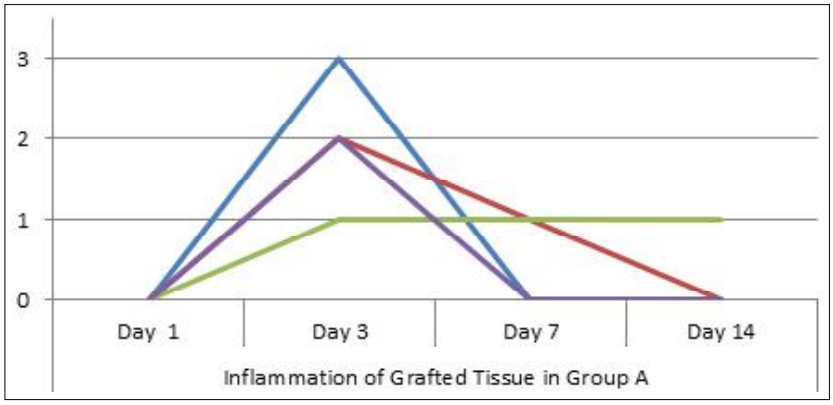

Fig 7: Inflammation of graft site group A.

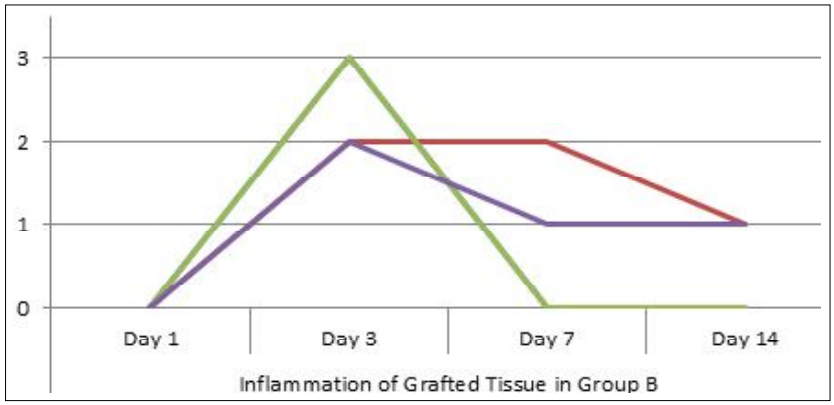

Fig 8: Inflammation of graft site group B.

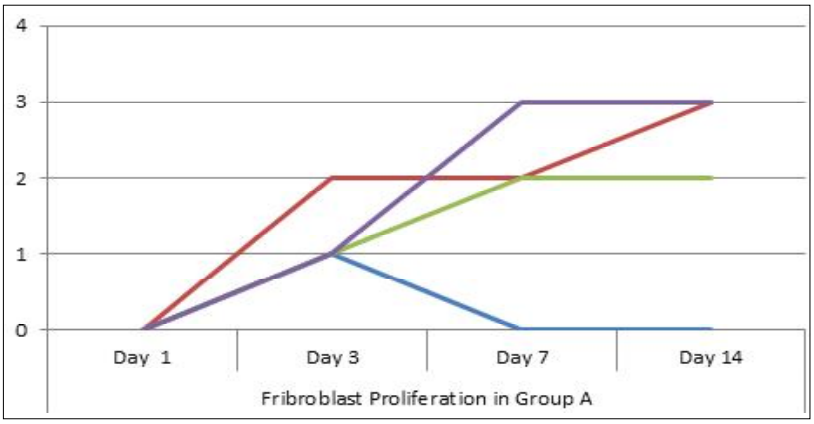

Fig 9: Fibroblast proliferation in group A.

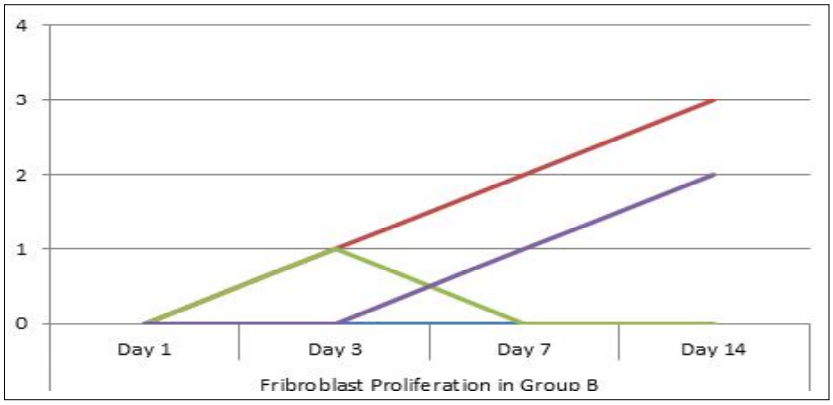

Fig 10: Fibroblast proliferation in group B.

present on margins of graft as well as in the surgical wound. This prevalence of granular tissue in wound was same as observed by Abegão *et al*. (2015). They conducted similar research in goats in which they found the wound stroma filled with granular tissue. Fibroblasts, blood vessels proliferation and macrophages were also observed by them. Fibroblast proliferation and formation on new blood vessels was found to be stimulated by the presence of macrophages.

CONCLUSION

On the basis of macroscopic and histo-pathological findings of this study it is concluded that autologous platelet rich plasma (PRP) is a better wound healing promoter as compared to homologous platelet rich plasma (PRP) with minimal post operative complications.

Conflict of interest: None.

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