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EVALUATION OF PLATELET RICH PLASMA (PRP) AND PLATELET RICH FIBRIN (PRF) FOR ENHANCING HEALING OF SKIN GRAFTS IN DOG

By

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ABSTRACT

The present work was designed to evaluate the effect of platelet rich plasma (PRP) and platelet rich fibrin (PRF) for enhancing graft acceptance and healing of autologous full thickness skin graft in dogs. Eighteen dogs were included in the study; dogs were divided into two groups: PRP-treated group and PRF-treated group. In all groups, the skin on both sides of the chest wall was prepared for the autogenous graft. Each side was used as donor and recipient for the opposite one. Two circumscribed full thickness skin incisions (4 cm in diameter) were harvested, one from each side, and implanted interchanged into the other side. Right side grafts were treated with either PRP (PRP-treated group) or PRF (PRF-treated group). The left side grafts were implanted without any treatment to act as self-control. Dogs were evaluated clinically on fourth, 7th, 10th, 14th, 18th and 21st day. Evaluation criteria included evaluation of coloration, exudation and cosmetic appearance. Histopathological evaluation was done on the 7th, 14th and 21st day (3 dogs from each group at each time point). Evaluation criteria included evaluation of re-epithelialization, inflammatory cell infiltration, granulation tissue formation, angiogenesis, fibroblast proliferation as well as collagen orientation. The results of the present study demonstrated that, the platelet concentration gel (PRP & PRF) were successfully used as a biological sealant for enhancing wound healing of the full-thickness skin grafts in dogs. PRF demonstrated promising results in enhancing graft acceptance compared with PRP. In conclusion, PRF provided a good and useful alternative in the management of reconstructive surgery and enhancing graft acceptance because it is autologous, safe, inexpensive and easily prepared.

Keywords:

Platelet-rich plasma (PRP); Platelet rich fibrin (PRF); skin graft; full thickness; dog.

INTRODUCTION

Reconstruction of large skin defects represents a major challenge for veterinary surgeons due to the lack of adequate loose skin required for primary closure. Skin grafts may be the only option for optimum restoration of structural and functional normality of the defect area **(White, 2009; Machail, 2013)**. Skin grafts may be either full thickness or split thickness grafts. The process of graft take comprises two separate but overlapping processes including adherence of the graft to the recipient bed and supplying adequate nutrition to the implanted graft. Adherence of the graft to the wound bed occurs by formation of fibrin network between the graft and the bed. The nutrition of the graft in the initial stages occurs by plasmatic imbibition from the recipient bed. Initially, healthy granulation tissue is the source of nutrition of the graft in this stage. Then the blood vessels within the graft begin to anastomose with the vessels of the recipient bed. This stage is termed as inosculation. In-growth of new vessels from the graft bed is responsible for further revascularization of the graft whereby time these vessels mature and differentiate into arterioles, venules and capillaries **(Swaim, 2003).**

Recent trends in cosmetic and reconstructive surgery are directed towards accelerating the healing without any complications. Platelet concentrates have been studied and applied in surgery for their wound healing properties. Platelets contain various growth factors that are released in a specific ratio and in a specific order on platelet activation. These factors are responsible for attracting inflammatory cells and fibroblasts and play a role in vascularization and enhancing the deposition of collagen **(Sclafani, 2009).** Platelet Rich Plasma (PRP) is an autologous platelet concentrate, which need anticoagulant and thrombin for preparation.

Platelet rich fibrin (PRF) is a second generation of PRP, which consists of platelets and leucocytes enmeshed in a complex fibrin matrix in a gel form without the addition of thrombin. This leads to incorporation of the platelet derived growth factors within the naturally formed fibrin mesh **(Dohan, Choukroun and Diss, 2006).** The fibrin mesh acts as a source of growth factors,which are released gradually over a prolonged period.

This sustained release of growth factors enhances the process of wound healing **(Sclafani, 2009).** The PRF matrix has also been shown to promote angiogenesis and fibroblast proliferation **(Sclafani and McCormick, 2012).** Promising results of PRP and PRF as tissue sealants in skin wound repair have been documented **(Eppley, Woodell and Higgins, 2004; Everts, Brown Mahoney, Hoffmann, Schönberger, Box, van Zundert and Knape, 2006)**,

while there are limited reports describing the role of PRP and PRF in enhancing graft take and acceptance in full thickness skin grafts. The aim of the present study was to evaluate the effect of PRP and PRF for enhancing graft acceptance and healing of autologous full thickness skin graft in dogs.

MATERIAL AND METHODS

Animals:

The present study was conducted on 18 adult Mongrel dogs of both sex (10 male and 8 female), aging 1.8± 0.7 (mean±SD) years, and weighing 16.4±2.6 (mean± SD) kg. During the study, dogs were kept in separate kennels, allowed free access to water, and fed twice daily. The Institutional Animal Use and Care Committee (IACUC) also approved the present study.

Preparation of Platelet Rich Plasma (PRP):

Collection of 17 ml blood was obtained from the cephalic vein using a syringe containing 3 ml of sodium citrate as anticoagulant. The blood sample was transferred into dry sterile tube (Falcon tube).Two centrifugations were required for PRP production.The initial centrifugation was performed at 2500 rpm for 4 minutes at room temperature resulting in two basic components: blood cell component in the lower fraction and plasma component in the upper fraction. The plasma component was decanted and re-centrifuged at 2500 rpm for 8 minutes resulting in two components: upper platelet poor plasma (PPP) and lower PRP. The PRP was separated from the PPP plasma component. Immediately before use, the PRP was activated by adding 200 IU of thrombin (Bovine plasma T4648, Sigma, USA) dissolved in 0.5 ml of 10% $CaCl_2$ solution (Calcium Chloride Anhydrous, Sigma, USA) to each ml of PRP and mixed well **(Mehrjerdi *et al* .,2008).**

Preparation of Platelet rich Fibrin (PRF):

Twenty ml. of blood was collected from cephalic vein. The blood was collected without anticoagulant in dry Falcon tubes then it were immediately centrifuged at 3400 rpm for 12 minutes at room temperature. After centrifugation, the PRF clot was removed from the tube using sterile tissue forceps and separated from the blood cell base using a sterile scissor **(Naik, Karunakar, Jayadev and Marshal, 2013)**.

Grafting Procedures:

All grafting procedures were carried out under complete general anesthesia, dogs were pre-medicated with atropine sulphate 1% (Atropine Sulphate®, El-Nasr pharm. Chem.Co.,

Egypt) at a dose of 0.05 mg/kg subcutaneously and tranquilized with xylazine HCL 2% (Xylaject®, ADWIA Co,. Egypt) at a dose of 1mg/kg intramuscularly 15 minutes before induction of anesthesia. General anesthesia was induced using ketamine HCl 10% (Ketamine®, Elyoser, Egypt) at a dose of 10 mg/kg and maintained with thiopental sodium 2.5% (Anapental®: Sigma-Tec, Egypt) in a guided repeated doses of 10 mg/ kg administered intravenous through canulated cephalic vein (dose to effect) **(Hall,Clarke and Trim, 2014).** Under complete aseptic conditions, two circumscribed full thickness skin incisions (4 cm in diameter) were harvested, one from each side. Then it was implanted interchangeably into the opposite site. In all dogs, the right-side grafts were used as the experimental grafts. Either PRP or PRF was used as a biological sealant, the left-side grafts were implanted without any treatment to act as self-control. After graft harvesting, the subcutaneous tissue was removed using scissors, until the hair follicles could be seen. The prepared skin grafts were placed into a sterile petri-dish containing sterile saline and gentamycin 80 mg (Garamycin®, MEMCO, Egypt) until implantation. One cm. longitudinal incision was conducted on each graft to provide adequate drainage. The prepared full thickness graft was placed on the recipient bed. Fixation of the graft was accomplished using absorbable suture material (Polycryl® 0 Egysorb, Taisier Med, Egypt) to oppose the graft edges with the surrounding skin of the recipient bed **(Swiam, 2003).**

In PRP-treated groups (n=9), the prepared PRP gel was placed as sandwich layer between skin defect bed before complete fixation of the grafted skin edges to the right-side recipient bed. In PRF-treated group (n=9), the prepared PRF gel was placed as sandwich layer between skin defect bed before complete fixation of the grafted skin edges to the recipient bed. The left side in all dogs was grafted without treatment to serve as a control.

Post-operative care:

Systemic antibiotic cephalosporin (Ceftriaxone®, Sandoz, Egypt) was given intramuscularly at a dose of 1gm/ 24 hours for 10 days. The grafts were protected with topical antibiotic combination of Gentamycin (Garamycin® 1%, Memphis Co., and Egypt) and Sodium fusidate (Fucidin® 2%, MINAPHARM, Egypt) was applied over the grafted wounds. A non-adhessive sterile dressing was applied evenly over the grafted surface, without wrinkles or folds. A thick padding with absorptive cotton layer was applied over the non-adhesive dressing then it was fixed in place using 2 covering stitches to maintain graft fixation and prevent its mobility. Chest bandage was applied using sterile gauze wrapped around the circumference of the chest

to cover the dressing of both sides. An adhesive tape was used to secure the bandage and prevent slippage. An Elizabethan collars was applied around the dog's neck to prevent licking and biting of the surgical wounds. The bandage was changed at 4th, 7th, 10th, 14th, 18th day following grafting.

Clinical evaluation:

The gross appearance of the skin graft was assessed on 4th, 7th, 10th, 14th, 18th and 21st day. Graft was evaluated based on color, cosmetic appearance, as well as exudation. Evaluation criteria included coloration:(black;pale; cyanotic;reddish; normal).Exudation:(severe exudate; moderate exudate; mild exudate; absence of exudate). Cosmetic appearance: (poor; fair; good; excellent).

Euthanasia and collection of tissue samples:

Dogs were euthanized at 7th, 14th, 21st day (three dogs from each group/ time point), using an overdose of thiopental sodium (100 mg/kg). Just immediate to euthanasia, tissue samples were collected from the site of grafting as well as the surrounding and underlying tissue.

Histopathological and immunohistochemical evaluation:

Tissue samples were fixed in 10 % neutral buffer formalin solution for 48 hours then embedded in paraffin, sectioned at 5µm thickness, and stained with hematoxylin-eosin (H&E) and Masson's trichrome (MT) **(Bancroft and Layton, 2012).** Tissue sections were stained cytokeratin AE1/AE3 high and low molecular weight keratins for immunohistochemical evaluation **(Erwin, Etriwati, Gunanti, Handharyani, Noviana, 2017).** Evaluation criteria included re-epithelialization, inflammatory cells infiltration, presence of dermal granulation tissue, angiogenesis, fibroblast proliferation, collagen orientation **(Gal, Toporcer, Vidinsky, Mokry, Novotny and Kilik, 2006).**

RESULTS

Clinical evaluation:

The clinical observations showed that most of the skin grafts of all groups took reddish to bluish-red (cyanotic) coloration during the first week, then changed gradually to reddish during the second week and regained their normal coloration within the third week post grafting, except in 3 control grafts were, one was pale and the others were black in coloration. Most of the skin grafts among the three groups showed mild to moderate exudation during the first 2 weeks, except two control grafts showed severe exudation.

Excellent cosmetic appearance was reached in two grafts. However, only on day fourteen and it was observed in fife of the treated grafts especially with PRF group during the third week post graft. Control group demonstrated poor (three grafts) to good (two grafts) while only one control graft showed excellent appearance on day 18 post grafting.

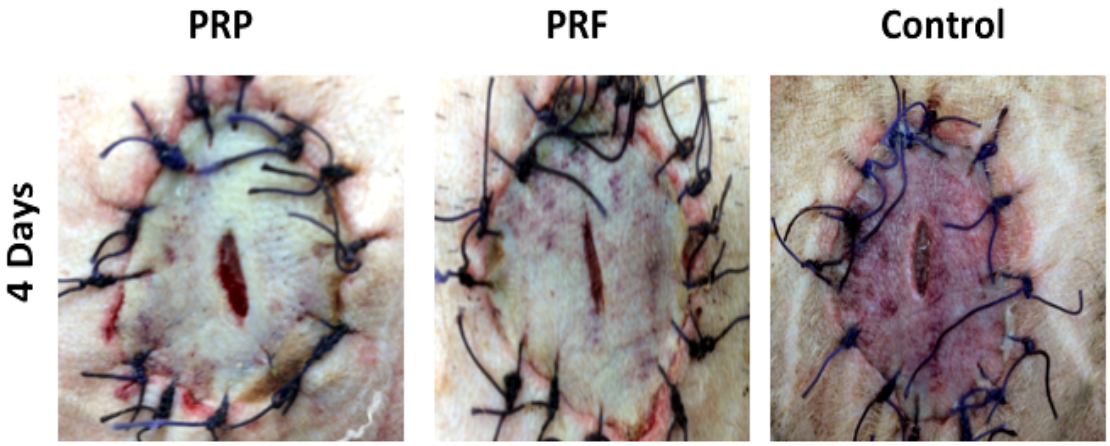

Fig. (1): Photograph of the PRP-treated and PRF- treated grafts compared to control graft at day 4 following graft. The control graft is more cyanotic than PRP and PRF grafts.

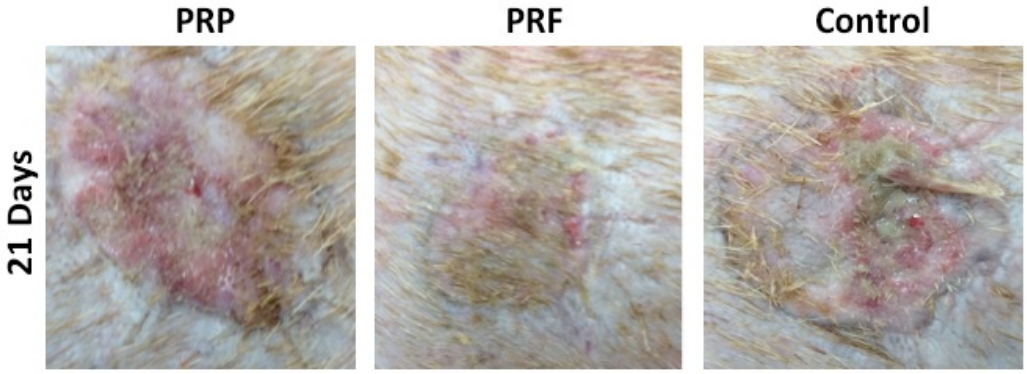

Fig. (2): Photograph of the PRP-treated and PRF- treated grafts compared to control graft at day21 following graft. Complete graft take with epithelialization of interstices and hair growth was best in PRF-treated grafts.

Histopathological evaluation:

Complete epithelialization of the interstices (union line) was observed in both PRP and PRF-treated groups on day 7, while re-epithelialization has not been completed in the control

grafts. The epithelialization was in progress with keratinization in the PRP and PRF groups on day 14, meanwhile, the control grafts had no or less keratinized epithelium.

Infiltration of inflammatory cells including neutrophils, plasma cells, macrophages and lymphocytes were present in all grafts, but with different degrees, since the amount of inflammatory cells on day 7 were higher in the control grafts compared with the treated grafts. By the day 14, the treated groups showed transient increase in the inflammatory cell followed by marked decrease on day 21 while the control grafts was still higher.

New vascularization was greater in both PRP and PRF groups compared with the control grafts, meanwhile PRF grafts showed more vessels compared with PRP group.

The density of granulation tissue of both treated groups was higher than that of the control grafts on day 7 and 14, followed by indistinct differences decreased on day 21 post-grafting. The fibroblast proliferation in the dermis region of the treated grafts showed marked increase on day 7 compared with the control grafts. On day 14, the fibroblast and the collagen fibers became denser, abundant and organized in both PRP and PRF groups than the control group. Moreover, on day 21, the proliferation of collagen fibers and collagen bundles were observed markedly higher in PRF-treated group compared to PRP and control grafts.

Immunohistochemistry demonstrated that cytokeratin AE1/AE3 expression takes 3 degrees of brown staining according to its density in the epithelial cells of epidermis and dermis layers of the skin. On day 7, cytokeratin was found with dark brown staining in the epithelial cells intracytoplasmic of PRF group compared to PRP group with brown staining and control grafts with light brown staining.

Histopathological finding of the autogenous full-thickness skin grafts of the PRP, PRF and Control groups at different evaluation times is demonstrated in Fig. (3 - 6).

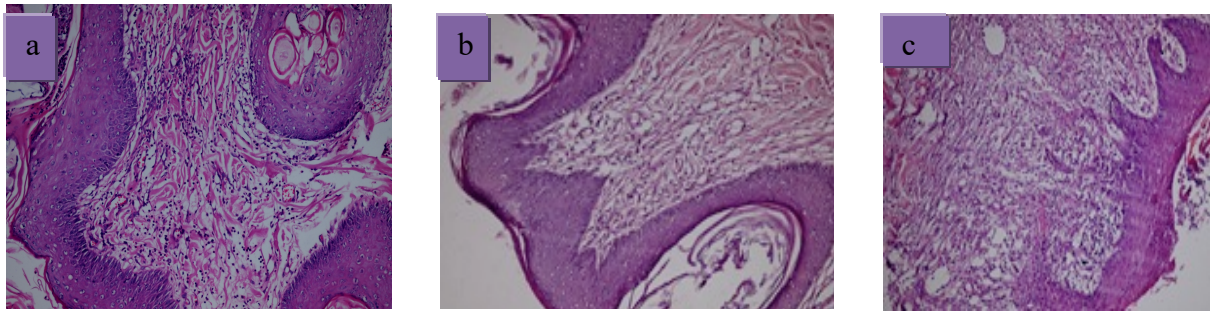

Fig. (3): On day 7, a) PRP group –Presence high amount of granulation tissue. b) PRF group- Presence high amount of granulation tissue. c) Control group - Presence of high amount of granulation,inflammatory cells and incomplete epithelialization. **H&E, X 200.**

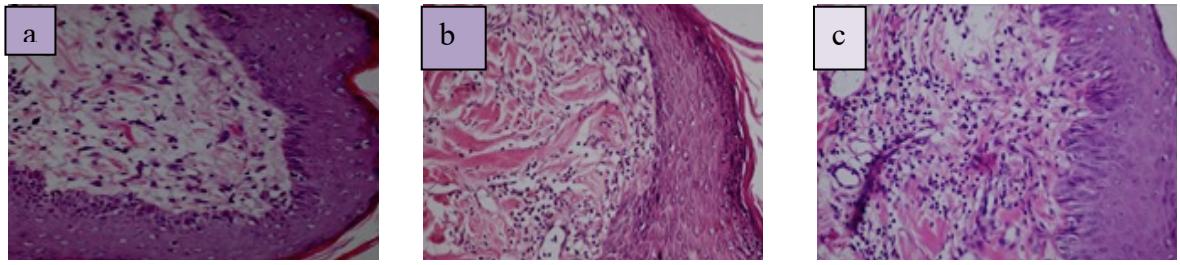

Fig. (4): On day 14, a) PRP group –, A good epithelialization with keratinized was seen in addition to infiltration of mononuclear inflammatory cells. **H&E, X 400. b)** PRF group A good epithelialization with keratinized was seen in addition to infiltration of mononuclear inflammatory cells.c) Control group-The epithelialization demonstrated with less keratinized epithelia in addition to infiltration of mononuclear inflammatory cells. **H&E, X 400.**

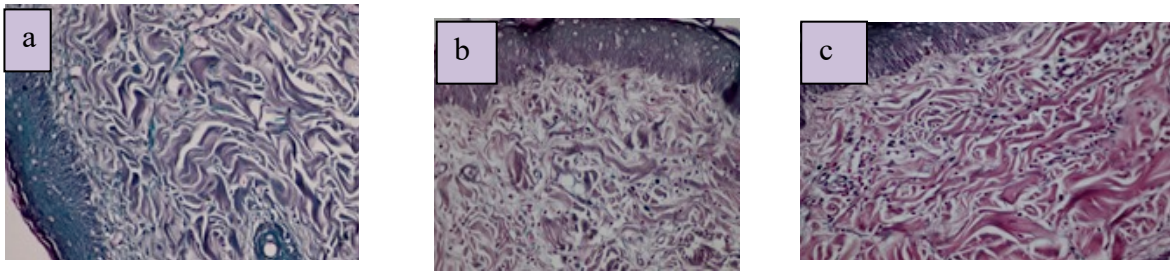

Fig. (5): On day 21, a) PRP group: Presence of large amount of collagen fiber bundles. b) PRF group: Presence of abundant amount of collagen fiber bundles. c) Control group: Presence of moderate amount of collagen fiber and mononuclear cell infiltration. Masson Trichrome, **X 400.**

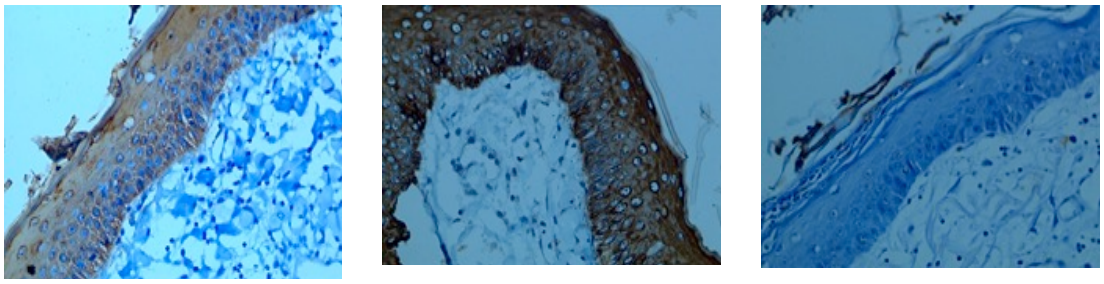

Fig. (6): On day 7, a) PRP group - Cytokeratin AE1/AE3 expression found as brown staining in epithelial Cell intracytoplasmic. b) PRF group - On day 7, Cytokeratin AE1/AE3 expression found as dark brown staining in epithelial Cell intracytoplasmic. c) Control - On day 7, Cytokeratin AE1/AE3 expression found as scant brown staining in epithelial Cell intracytoplasmic. **IHC, X 400.**

DISCUSSION

Results of the presented study demonstrated that, the platelet concentration gel (PRP & PRF) were successfully used as a biological sealant for enhancing wound healing of the full-thickness skin grafts in dogs. PRF demonstrated promising results in enhancing graft acceptance because it is autologous, safe, inexpensive, and easily prepared. Skin grafts are indicated for reconstruction of large skin deficits where there is lack of adequate surrounding loose skin for primary closure **(Aisa and Vernon, 2016)**. Free grafts are, sometimes, the only option for optimum restoration of structural and functional normality of the affected area. The reddish and bluish-red (cyanotic) coloration of the grafted skin observed in the first week are good sign for graft acceptance as they denote early revascularization of the graft **(McKeever and Branden, 1978)**. The graft survives by imbibitions through which the skin soaks up proteinaceous fluid (serum) from the wound bed, which provide oxygen, and nutrients during the first 2 days after graft **(Swaim, 2003)**. This was followed later on by inosculation and connection of capillaries in the wound bed with the cut vessels of the skin graft that take place from day 4 to 7 after graft. Avascular necrosis encountered in the control group was indicated by persistence of pale skin graft, while dry ischemic necrosis appeared as a black discoloration **(Cleay, 2016).** Most skin grafts showed mild to moderate exudation during the first 2 weeks, except two cases of the control grafts showed severe exudation. The dry nature of the grafted site without exudation observed in approximately all treated grafts during the third week (14 - 21 days) post grafting which were considered signs of

terminal stage of healing **(Swaim, 2003)**.On day 7,complete epithelialization of the interstices was noticed in both treated groups, while re-epithelialization has not been completed in the control grafts. The epithelialization was in progress with keratinization in the treated groups on day 14, meanwhile, the control grafts had no or less keratinized epithelium. These findings are in consistent with earlier reports suggesting that PRP gel has its greatest effect on healing on 14 day after grafting **(Hom, Linzie and Huang, 2007)**. Application of autologous PRP using subcutaneous infiltration route at the wound margin of dogs showed significant enhancement of wound re-epithelialization and reduced scar formation **(Farghali, Abd ElKader,Khattab,Abu Bakr,2017).** New epidermis layers formation during wound recovery did not take place without the help of growth factors, cytokine, extracellular protein matrix and local materials that are secreted during inflammation by fibroblast endothelial cells, and keratinocyte **(Moll,Houdek,SchmidtandMoll,1998)**. Regarding histopathological assessment of the present study, it was evident that, infiltration of inflammatory cells including neutrophils, plasma cells, macrophages and lymphocytes which were present in all grafts, but with different degrees, since the amount of inflammatory cells on day 7 were higher in the control grafts compared with the treated groups. However, by day 14, the treated groups showed transient increase in the inflammatory cell followed by marked decrease on day 21 while the control grafts was still higher.Similar observations were reported by **Farghali *et al.*, (2017)** who noticed that, the number of infiltrating polymorph nuclear leukocytes were increased in both control and PRP treated wounds in the second week compared with the first week. The transient increase of the inflammatory cells in the treated groups during the second week may be due to the growth factors of the PRP promote cell proliferation, differentiation chemotaxis and induced the migration of various cells preferably macrophages and myofibroblast, which initiate tissue repair and regeneration **(Barrientos,Stojadinovic, Golinkos,Brem and Tomic-Canic, 2008)**. As it happened in the present study, a single application of PRP in the incisional wound amplified the inflammatory response with an increased wound influx of neutrophils and macrophages **(Gokulakrishnan, Nagarajan, Ramani, Akannan and Safiuzamma, 2016).**Regarding angiogenesis of the grafted skin,the histopathological examination showed that, the degree of new vascularization was greater in both treated groups compared with the control grafts, meanwhile PRF-treated group showed higher vascularization than PRP-treated group. These findings were coincided with the clinical observation as they explain the color change of the skin after grafting. Wound healing of skin grafts is different from other wounds

as it completely dependent on revascularization or inosculation of the graft. The increased size and number of blood vessels are among the factors important for successful graft wound healing, because blood vessels supply oxygen and nutrients to the tissue undergoing regeneration. Various factors are known to induce angiogenesis **(Schilling, Favata and Radakovich,1953)**. Among these factors vascular endothelial growth factor (VEGF) which is the most powerful angiogenic cytokine secreted from platelets, macrophages and fibroblasts. The release of VEGF stimulates endothelial cell proliferation which assists wound healing **(Anitua,Andia, Ardanza,Nurden andNurden,2004)**. Platelet-derived growth factor (PDGF) contained in PRP may be a key factor in tissue regeneration, mediating the regulation of cell growth and division, as well as angiogenesis and the growth of pre-existing blood vessels **(Bennett and Schultz, 1993;Stellos and Gawaz, 2007)**. In the present study, the density of granulation tissue of both treated groups was higher than that of the control grafts on day 7 and 14, followed by indistinct differences decreased on day 21-post graft. The fibroblast proliferation in the dermis region of the treated grafts showed marked increase on day 7 compared with the control grafts. On day 14, the fibroblast and the collagen fibers became denser, abundant and organized in both of the treated groups than the control grafts. Moreover, on day 21, the proliferation of collagen fibers and collagen bundles were observed markedly higher in PRF-treated group compared with PRP and control groups.Considering the functions of the major PDGF, it is a potent chemo-attractant and mitogen for fibroblasts that stimulates collagen synthesis and angiogenesis by growth of pre-existing blood vessels **(Rozman and Bolta, 2007;Alsousou,Thompson, Hulley, Noble and Willett,2009)**.

The collagen is a major extracellular matrix constituent required for maintenance of skin tensile strength and elasticity. It has been reported that the increase of wound tensile strength that take place during fibroblastic phase corresponds to the increased level of collagen wihin the wound **(Broughton and Rohrich, 2005)**. Immunohistochemistry evaluation of the present study demonstrated that cytokeratin AE1/AE3 expression takes 3 degrees of brown staining according to its density in the epithelial cells of epidermis and dermis layers of the skin. On day 7, cytokeratin was found with dark brown staining in the epithelial cells intracytoplasmic of PRF group compared to PRP group with brown staining and control grafts with light brown staining. In addition, it showed dark brown staining in epidermis layer epithelial cell and light brown on dermis layer of PRF-treated group. Cytokeratin is an

intermediate filament keratin protein found in epithelial cytoplasm and has an important role in characterizing cell differentiation.

CONCLUSION

The present study proved that, autologous platelet concentration (PRP and PRF) in gel form are essential for enhancing graft acceptance in autogenous full thickness skin graft in dogs. PRF presented results that was clinically and histopathologically more efficient than PRP when use as biological sealant for enhancing wound healing. Single applications of the freshly prepared gel together with good fixation, effective drainage, and good fixation and without infection were sufficient for successful graft survival and acceptance.

REFERENCES

Aisa, J. and Vernon, J. (2016): The use of skin grafts in small animal reconstructive surgery. Companion Animal, 11 (21), pp. 642-648.

Alsousou, J., Thompson, M., Hulley, P., Noble, A. and Willett, K. (2009): The biology of platelet rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br, 91(8), pp. 987-996.

Anitua, E., Andia, I., Ardanza, B., Nurden, P. and Nurden, A.T. (2004): Autologous platelets as source of proteins for healing and tissue regeneration. Thromb Haemost, 10 (1), pp. 4-15.

Bancroft, J. D. and Layton, C. (2012): The hematoxylins and eosin. In: S. K. Suvarna, C. Layton, and J. D. Bancroft, eds., Bancroft Theory and Practice of Histological Techniques, 7Th ed. New York, Churchill Livingstone, pp. 173 -187.

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H. and Tomic-Canic M. (2008): Growth factors and cytokines in wound healing. Wound Repair Regen, 16 (5), pp. 585-601.

Bennett, N. T. and Schultz, G. S. (1993): Growth factors and wound healing: part II. Role in normal and chronic wound healing. Am J Surg, 166 (1), pp. 74 - 81.

Broughton, G. and Rohrich, R. J. (2005): Wounds and scars. SRPS, Part I. 7 (10), pp. 1-54.

Claeys, S. (2016): Skin Grafting. In: D. Griffon, A. Hamaide, eds., Complications in Small Animal Surgery, Oxford, Willy Blackwell, pp. 561-568.

Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J. and Gogly, B. (2006) [a]: Platelet-Rich Fibrin (PRF): A second generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 101 (3), pp. E37- 44.

Eppley, B. L.; Woodell, J. E. and Higgins, J. (2004): Platelet Quantification and Growth Factor Analysis from Platelet-Rich Plasma: Implications for Wound Healing. Plast Reconstr Surg, 114 (6), pp. 1502-1508.

Erwin, Etriwati, Gunanti, Handharyani, E., Noviana, D. (2017): Changes in histopathology and cytokeratin AE1/AE3 expression in skin graft with different time on Indonesian local cats. Vet World, 10 (6), pp. 662 - 666.

Everts, P. A., Brown Mahoney, C., Hoffmann, J. J., Schönberger, J. P., Box, H. A., van Zundert, A. and Knape,J.T.(2006): Platelet-Rich Plasma Preparation Using Three Devices: Implications For Platelet Activation And Platelet Growth Factor Release. Growth Factors, 24 (3), pp. 165-171.

Farghali, H. A., Abd-El-Kader, N. A., Khattab, M. S. and AbuBakr, H. O. (2017): Evaluation of subcutaneous infiltration of autologous platelet-rich plasma on skin-wound healing in dogs. Biosci Rep, 37 (2), pp. 1-13.

Gal, P., Toporcer, T., Vidinsky, B., Mokry, M., Novotny, M. and Kilik, R. (2006): Early changes in the tensile strength and morphology of primary sutured skin wounds in rats. Folia Bio, 52 (4), pp. 109-115.

Gokulakrishnan, M., Nagarajan, L., Ramani, M., AKannan, T. and Safiuzamma, M. (2016): Comparison of Histological Changes of Recipient Wound Bed by Platelet Rich Plasma and Adipose Derived Stem Cell in Dogs. IJSR, 5 (11), pp. 247-252.

Hall, L. W., Clarke, K. W. and Trim, C. M. (2014): Anaesthesia of the dog. In: Veterinary Anaesthesia, 11th ed. London, W. B. Saunders, pp. 405 - 498.

Hom, D. B., Linzie, B. M. and Huang, T. C. (2007): The healing effects of autologous platelet gel on acute human skin wounds. Arch Facial Plast Surg, 9 (3), pp. 174 -183.

Macphail, C. M. (2013): Surgery of the Integumentary System. In: T.W. Fossum, Small Animal Surgery, 4th ed. Philadelphia, Mosby Elsevier, pp. 190 -278.

McKeever, P. J. and Braden, T. D. (1978): Comparison of full- and partial-thickness autogenous skin transplantation in dogs: a pilot study. Am J Vet Res, 39 (10), pp. 1706 -1709.

Mehrjerdi, H. K.; Sardari, K.; Emami, M. R.; Movassaghi, A. R.; Goli, A. A.; Lotfi, A. and Malekzadeh, S. (2008): Efficacy of Autologous Platelet-Rich Plasma (PRP) Activated By Thromboplastin-D on the Repair and Regeneration of Wounds in Dogs. IJVS, 4 (3), pp. 19-30.

Moll, I.; Houdek, P.; Schmidt, H. and Moll, R. (1998): Characterization of epidermal wound healing in a human skin organ culture model: Acceleration by transplanted keratinocytes. J. Invest. Dermatol, 111 (2), pp. 251-258.

Naik, B., Karunakar, P., Jayadev, M and Marshal, V. R. (2013): Role of Platelet rich fibrin in wound healing: A critical review. J Conserv Dent, 16 (4), pp. 284 -293.

Rozman, P. and Bolta, Z. (2007): Use of platelet growth factors in treating wounds and soft-tissue injuries. Acta Dermatovenerol Alp Pannonica Adriat, 16 (4), pp. 156-165.

Schilling, J. A., Favata, B. V. and Radakovich, M. (1953): Studies of fibroplasia in wound healing. Surg Gynecol Obstet, 96 (2), pp.143-149.

Sclafani, A. P. (2009): Applications of Platelet-Rich Fibrin matrix in facial plastic surgery. Facial Plast. Surg, 25 (4), pp. 270-276.

Sclafani, A.P. and McCormick, S.A.(2012): Induction of dermal collagenesis, angiogenesis, and adipogenesis in human skin by injection of Platelet- Rich Fibrin matrix. Arch. Facial Plast. Surg, 14 (2), pp. 132-136.

Stellos, K. and Gawaz, M. (2007): Platelet interaction with progenitor cells: potential implications for regenerative medicine. Thromb Haemost, 98 (5), pp. 922-929.

Swaim, S. F. (2003): Skin Grafts. In: D. H. Slatter, ed., Textbook of Small Animal Surgery, Philadelphia, Elsevier Science, pp. 321-338.

White, R.S. (2009): Skin grafting. In: J. Williams and A. Beck, eds., Manual of canine and feline wound management and reconstruction, 2nd ed. Hampshire, Fording Bridge, pp. 82-94.