Impact of Platelet Rich Fibrin Derived From Peripheral Blood and Bone Marrow (Solid Bone Marrow Aspirate Concentrate) on Skin Autograft Healing in Dogs: Comparative Study

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Abstract

Platelet rich fibrin (PRF) derived from peripheral blood (pb-prf) is the one of most new and commonly utilized platelet concentrate in medical therapeutic field. It has an essential role in tissue repair and healing as it is a source for a wide range of different types of growth factors, anti-inflammatory and post-inflammatory molecules. On the other hand, recently bone marrow has been used as a source for PRF to accelerate wound healing instead of arterial or venous peripheral blood source. This experiment was carried out to study the effect of bone marrow derived PRF, which also called solid bone marrow aspirate concentrate (sBMAC) on full thickness skin autograft take, incorporation, survival and healing, and also to compare its influence with effect of pb-prf. Our current study comprised 27 dogs in which skin autografting were done, and were divided into three groups. A control group, in which graft was carried out without any treatment; pb-prf group, in which graft was treated with PRF derived from peripheral blood and sBMAC group, in which graft was treated with PRF derived from bone marrow.

The site of grafting was inspected macroscopically during 28 days after graft implantation. The histopathological assessment for grafting area was achieved at 7th, 14th and 28th days after operation. Results showed that the two types of PRF had a stimulation impact in skin graft incorporation with grafted wound bed and healing, that was characterized by 100% graft acceptance, excellent cosmetic graft appearance, enhancement of granulation tissue creation and maturation, faster reepithelization, better graft survival in contrast to control group, which displayed a partial graft separation and late granulation tissue maturation, reepithelization and healing. Besides that, the sBMAC graft treatment had shown a better healing outcome during the all phases of the graft healing process in comparison with the pb-prf. In conclusion, the sBMAC had a greater effectiveness than the pb-prf in full thickness skin autograft healing.

Key words: PRF, bone marrow, , Skin grafts.

Introduction

A skin graft is a cutaneous segment that is free separated from the donor site and transferred to be transplanted it in a recipient position. Skin grafts are used when healing by primary wound closure; second intention and flap restoration are deemed inappropriate [1]. They are suitable to treated large defects resulted after many injuries as burns, sever trauma and tumor excision. They provide a suitable choice for resurfacing cutaneous wound and providing a supporting bed for the quicker tissue regeneration and healing. Additionally, the skin grafts act as a barrier for preventing entrance of external microorganisms and waste product [1-3].

Grants according to thickness are classified to a split thickness or full thickness [4]. In dermatology, the most common efficaciously used grafts are full thickness grafts which characterized by excellent skin takes and match to the host site. As well as after repairing, it is healed with minimal shrunken, scarring and have texture, hair growth, color and elasticity similar to normal skin, unlike the split-thickness grafts that have multiple disadvantages including removal of subcutaneous tissue and non-viability areas is tedious and planning somewhat difficult [1,5]. On the other hand, in regard to donor sites source, skin grafts are categorized as autograft, isograft, allograft and xenograft. The prominent being used autogenic grafts, which are considered the best standard option for wound repair care. It is...
harvested from one portion of the body and transplanted to another in the same person and it is characterized over the all graft by it is not submitted to host immune rejection [1-3]. Graft viability and survival depends on preventing affection, excellent graft immobilization for crucial graft revascularization and minimal seroma and hematoma accumulation. Hematoma, seroma, bad vascularization of recipient bed and infection are considered the main complications that maybe reduce the chance of graft survival then breakdown and subsequent failure [1-6].

Failure of the graft to be accepted and heal can lead to repetition of grafting process which in turn makes the surgery and medical therapy more expensive with addition to increase the hospitalization time [6]. As a result for these reasons, the researchers directed to using different materials and techniques aimed to accelerate and improve skin graft healing and reduce all complications and obstacles associated with healing process that lead to skin graft failure by reducing hematoma and seroma accumulation, enhancing vascularization, increasing contact between recipient wound bed and graft, as well prevention and elimination the contamination [6-9]. One of the most important ideal therapy modality used for this purpose is the biomaterials and platelet concentrates which derived from peripheral blood either venous or arterial like platelet rich plasma and platelet rich fibrin [6,7].

From the earliest reports nearly more than 20 years ago when the PRF was first launched, and since then, it has been used in regenerative medical therapy, it is use has gained pervasive acceptance across many fields of medicine [10-13].

It has been historically established that, the PRF use being associated with a high success rate in the adherence of skin graft and biological wound dressing materials on wound beds, since it improves scar tissue outcomes and has influences on the establishment of blood supply in the recipient site and enhancement tissues rejuvenating [10-12,14-16]. It has been used as a regenerative materials in dermatology in different types from multiple sources, including autologous, homologous and heterologous source[7,10,11]. The positive effect of fibrin on skin regeneration is due to its ability to provide the tissues with large amounts of growth factors for long periods of up to 14 days, that play promising effect in enhance tissue regeneration, neo-vasculogenesis and bacteriostatic, in addition to their ability to release enzymes and other biological immune mediators substances such as cytokines and chemokines which are necessary to prevent inflammation and infection and stimulate regeneration [13,16].

Previously, PRF is prepared during surgery from peripheral blood either venous or arterial because it is easy to obtain even in awake patients, and it was observed that there is no statistical difference between them. Recently, PRF derived from bone marrow has been developed in the surgical field to treat many tissue lesions [17] Fully-grown bone marrow has been assessed as a useful donor tissue site for regenerative cell therapy for different body tissue repair such as the skin, since it consist form hematopoietic elements and mesenchymal stem cells[18,19]. There is a collection of stem cells type in the adult body tissues that can differentiate in to specific cell types same to that tissue only, but the hematopoietic stem cells derived from bone marrow can generate and differentiate not only to cells similar to their origin but also into other cell types present in other body tissues [20]. Nishimoto et al., 2017 plus Koyanagi et al., 2022 displayed a method to prepare PRF from bone marrow through a harvesting protocol similar to peripheral blood derived PRF without addition any anticoagulants. Regardless to that sBMACs produce in less quantity than the other two types produced from peripheral blood, it provides a high quantity of regenerative hematopoietic, mesenchymal stem cells and wide variety types of growth factors, which is believed that lead to high tissue regeneration [17,21]. Koyanagi et al., 2021 conducted a study in vitro in which they compared the effect of sBMACand those PRF that derived from peripheral blood on osteoblast viability, migration capability, genetic material expressions, collagen synthesis, regeneration and mineralization potential. They were noted that sBMAC had a greater and faster effect on osteoblast differentiation, migratory potential, reproduction and bone tissue formation [17]. Also Uno et al., 2022 studied the effect of sBMAC on the rotator cuff tendon tearing repair and compared its effect with the effect of platelet rich plasma, and they hypothesized that sBMAC enhanced healing of tendon-bone connection and maturation faster than other groups and the level score of growth factor VEGF was higher than other groups too [22].

Peripheral blood derived PRF has been used for skin graft healing and resulted in enhancement and acceleration of graft acceptance and healing [7,11,12]. On the other hand, bone marrow derived PRF (sBMAC ) might be more useful than pb-prf in the skin graft healing because the quantity of growth factor and cytokine that contains is different from the arterial and venous derived types. According to latest data there is no study reported on the usage of sBMAC on skin graft healing. Therefore, this study aimed to investigate the effect of sBMAC on skin graft take, integrity and healing, and to compare their effect with pb-prf effect.
Material and Methods

Pb- prf-and sBMAC Preparation

A volume of 10ml of venous blood was taken from jugular vein and 10 ml of bone marrow was collected from femur bone by using Jamshidi needle (Figure 1). The samples of peripheral blood and bone marrow were transferred into centrifuge tubes without addition any anticoagulant, and immediately at room temperature they centrifuged for 10 min at 3000 rpm [12,17, 21]. After centrifugation, three layers were formed in the tube, the middle layer embodied the PRF clot components, which was isolated from the tube using a sterile forceps (Figure 2 (A and B)), and then they were detached from the attached layer joined to it by means of scissors.

Animals

In our experiment, twenty-seven adult dogs from local breeds were used; their weight and age average (24±0.4) kg, (2.5±0.8) years respectively. Animals’ health was checked and were all housed in at same condition in cages separately. The experimental animals were split randomly into three groups: control, pb-prf and sBMAC.

Surgical procedure

All animal were anaesthetized under protocol of general anesthesia using mixture of Ketamine 10% and Xylazine hydrochloride 2%, which intramuscularly injected at a dose 3 mg/ kg B.W. and 10 mg/ kg B.W. respectively after administration of atropine sulfate as a pre-anesthetics drug subcutaneously at does (0.04mg/kg). Once the animals were anaesthetized, its skin back at both left and right side of vertebral column area was prepared according to the fundamentals of surgery. The operation began by making a full thickness excisional wound of 4x4 cm2 in size in the left side of back, and then a same sized section of skin was harvested from the right side of back (Fig.3: A) and implanted in the excisional wound site, then the graft secured to recipient site with interrupted suture technique using silk suture material (Figure 2 (A and B)). After that, the experimental animals were divided into 3 groups (9 for each). First as control; in which the grafted place left without any treatment, second; pb-prf treated group and third; sBMAC treated group. In both second and third groups, before sew up the latest two stiches, the pb- prf and sBMAC clot was introduced beneath the graft, then the sew technique was completed (Figure 3:D). The skin graft for all dogs was protected in place by non-adhesive pressure dressing for five days and antibiotic penicillin (10000 IU) and streptomycin (10 mg/kg) were received intramuscularly for 5 consecutive days.

Assessment of healing

The evaluation of the graft healing results was conducted through monitoring the gross changes in addition to histopathological changes of the grafted site at the 7th, 14th and 28th post-grafted days.

Gross assessment

Animals were assessed macroscopically daily to examine and monitor graft rate take, infection, allergic reaction, seroma formation, weeping occurrence and graft survival was estimated at day 7th, 14th and 28th post graft implantation through evaluation the skin graft color changes from photographic documentation using digital camera. The survival ratio (color alterations in skin graft) scored through the following criteria which mentioned in table-1 [23]. Cosmetic appearance of graft was graded according to table- 2 [24]. Also scar tissue formation was observed at the end of study.

Histopathological assessment

The grafting area samples of different period of treatment 7, 14 and 28 days post-graft transplantation were harvested, fixed in (10% formalin) for 5days, routinely processed and embedded it in paraffin and sectioned at thickness 5 mm thick, then de-paraffin zed by immersion it in xylene, and stained using standardized histological procedures with hematoxylin and eosin (H&E). All histological sections were assessed by pathologists and photographed by microscopic camera.

The assessment main histopathological outcome involved the inflammatory cell infiltration, amount of granulation tissue formation and maturation, re-epithelialization and angiogenesis. The histological events were scored built on the scoring system suggested by Arslantaş et al., [25], table- 3.

Statistical analysis

The package for sigma plot software program was used for the statistical analysis. Data of study was presented as mean and standard error (mean ±S.E.). One-way ANOVA was used to compare various groups, while Duncan’s multiple comparison tests was utilized to compare within a period intervals. Chi- square analysis was employed for assessment the skin graft take and weeping occurrence data between three groups .The final results were considered statistically significant at a threshold P < 0.05. Microsoft excel was used to explains the final data as a diagram.

Result

Gross observations

Out of 9 grafted wounds in the first group, two animals had partial loss of graft at a rate of (22.2%), and (7) had complete graft take at ratio (77.8%), whereas, there was no total loss of the graft. While
the 2nd and 3rd groups had a higher percentage of graft take with 9 cases out of 9 (100%) for each as compared with first group which had 7out of 9 animals and the animals did not show partially or completely graft loss (Fig. 4).

Seroma formation during the 4th day after grafting was observed in 7 out of 9 (77.8%) in control group and 3out of 9 grafts (22.2%) in pb-prf group, while in sBMAC was observed in one animal (11.1%). In 7th day the seroma was observed only in control group in 3out of 9 cases (33.3%). In all groups, the wound fluid color was pink to brown, its quantity was small, and it had a serosanguineous texture (Figure 5). On the other hand, weeping occurrence rate of skin graft was high noticeable in G1 than in pb-prf and sBMAC groups specially at the first 7th days after graft implantation, it was noted in one out of 9 animals (66.6%), whereas in pb-prf and sBMAC groups was seen in 2 out of 9 (22.2%) and 1 out of 9 (11.1%) grafted animal respectively (Fig. 6).

The changes in skin graft color to color similar to normal healthy skin (skin graft survival score) at 7th days after grafting in control group was significantly higher than in pb-prf-m and ± sBMAC groups (3.2± 1.0, 2.4± 0.0 and 2.3±0.6 respectively). In day 14th after grafting, there were progressive gradual changes in graft color and the score of graft survival in all groups declined, but the lowest score was in the sBMAC group, followed by the pb-prf group and then the control group (1.7± 0.0, 2.0± 0.3 and2.6± 1.2 respectively). whereas on day 28th after grafting the color of graft in sBMAC group was similar in appearance to original skin and the graft survival score was lowest and significantly differed from control group but there was no significant difference between it and pb-prf group( Table 4; Fig.7).

When taking into consideration the cosmetic appearance of the grafted area at the end of study, the skin transplanted in the sBMAC and pb-prf groups showed superiority upon the group control that characterized by good to excellent cosmetic appearance of graft, whereas transplanted area of control groups showed reasonable to poor appearance. Grafted area treated with either pb-prf- m or bone marrow derived PRF showed least or absent scar tissue formation after healing when compared with control group which showed more scar formation. Also at the end of study (28th days after grafting operation), hair grew were better in sBMAC group than in groups 1 and 2 (Fig. 8).

Histopathological assessment

As shown in (Fig.9), the histopathological section derived from the control group at the day 7th post skin grafting was characterized by presence of high inflammatory cells infiltration (score 3) with fibrinous exudate infiltration, in addition to presence of epithelium destruction with slight re-epithelialization and high granulation tissue formation (score 3). At the day 14th post skin graft implantation, the inflammatory exudative cells persisted which contained inflammatory cells infiltration (score 2), also fibrinous exudate, granulation tissue (score 2), hyperkeratosis and re-epithelialization (score 2) were observed (Fig.10).Where at day 28th after graft implantation histological feature revealed granulation tissue creation (score 2) distinguished by neovascularogenesis and collagen fiber formation with mild inflammatory cells infiltration (score 1) re-epithelialization (score 3) and hyperkeratosis surrounding by epithelial cells (Fig. 11).

Histological section for the pb-prf group at day 7th after graft implantation (Figure 12) showed presence of inflammatory crust comprise inflammatory cells (score 2), fibrinous exudate, granulation tissue formation (score 2), hyperkeratosis, re-epithelialization (score 1). In day 14th after graft implantation (Fig.13), the inflammatory cell infiltration was decreased (score 0) as compared with the previous period with presence of granulation tissue (score 2), hair follicle formation (HF), hyperkeratosis, complete re-epithelialization (score 3). While in 28th days, the histological section revealed to disappearance of inflammatory cells infiltration (score 0), increase in granulation tissue development but was still in (score 2), there were wide areas of neo-collagenases and angiogenesis (score 2), complete re-epithelialization with thick epithelium (score 3), formation of hair follicle, and sebaceous gland (Fig.14).

The microscopical examination of skin grafting area section from the sBMAC group generally in comparison with other groups (control and pb-prf) resulted in best outcome for the autograft skin repair and healing. Where at day 7th (Fig.15), showed absence of inflammatory cells infiltration(score 0), granulation tissue formation (score 2), re-epithelial formation (score 2) and angiogenesis (score 1).while at 14th days (Fig.16), showed also the wound between skin graft and original skin without inflammatory cells infiltration (score 0), granulation tissue formation with sever progress in its maturation (score 3) with high angiogenesis (score 2), complete re-epithelialization with thick epithelium (score 3). Where in last study period (day 28th), the histopathological examination showed also disappearance of inflammatory cells infiltration (score 0), complete maturation of all granulation tissue (score 0), with complete re-epithelialization with thick epithelium (score 3), and normal architecture as hair follicle, sweat gland and sebaceous gland (Fig.17).
 IMPACT OF PLATELET RICH FIBRIN ON SKIN AUTOGRAFT HEALING IN DOGS 1581

Discussion

Naturally the normal healthy body has ability over time to resolution and repair uncontaminated simple wounds, but on the other hand, patients with extensive skin defects caused by burn, tumor resection and severe trauma may require extensive treatment with using grafts, one of them is the autografts which are considered the golden basic standard for implantation [1,3]. Once the skin graft incorporated with original skin, it provides the wounds with safeguard from the pathogens, environment, in addition to protect it from unwanted temperature and excessive water loss similar to normal skin [26]. Full thickness skin graft is solitary of the very important grafts intended for restructuring defects in skin, it's usually heal with excellent functional and aesthetic results, but require abundant nourishment [27]. Generally, the success of skin graft take and healing depend on several factors, one of them comprising those factors related to the grafted area itself such as blood nourishment from underlying tissue, microbial infection and the appropriate dressing [1,4,5].

On the other hand, the recuperating process of skin grafts comprises highly diverse dynamic interaction of molecular and cellular events that result in dermal reconstruction, re-epithelization and skin remodeling. Principally, various essential particles as cytokines, chemokines, extracellular matrix molecules and growth factor at the injury site interact with numerous cells activities for stimulation and modulation it, that result in progress and improve the skin healing [12,18,28]. Over the past decades numerous scientific researches have been employed to use ingredients to support and promote the fixation, cohesion and healing of skin graft in the recipient place with a special focus on the use of biomaterials therapies derived from natural biological sources as PRP and PRF [6,11,12,15,18].

PRF has been frequently used as a biologic derivative in many therapeutic medicinal fields principally in maxillofacial, intestinal, ophthalmic and plastic surgery, in addition to dermatology field too[11,12,14,29,30,31]. In 2001 Choukroun et al. was technologically developed this biological fibrin products by using a simple method without addition any exogenously matters [13]. It's played an important role in tissue cells migration, proliferation and regeneration, its releases significantly during more than seven days large quantities of important healing molecules as cytokines, chemokines and growth factors as Vascular Endothelial Growth Factor [32]. So, it exemplifies one of the most important biomaterials used in regenerative therapy, because it is self-obtained with low cost and provides a high rate of tissue regeneration. In addition, it doesn't have any contraindications [11,13,14].

Skin grafting failure is occur by multiple causes, including: Firstly, hematoma or seroma formation beneath a graft that detaches the graft from the wound bed and disrupts revascularization. Secondly, infection that creates exudative fluid which separates the graft from wound bed. In addition, the enzymes in exudates digest the fibrin clot that is play role in holding the graft in recipient site, further impairing revascularization and cause skin graft loss too [1,3-5]. In our current, PRF either from peripheral blood bone marrow prevented formation the hematoma, seroma and infection. This is due to the adhesive, anti-inflammatory and anti-inflammatory characteristics of the fibrin matrix [30-34].

Generally, the therapeutic benefit of PRF in the skin graft survival is perhaps built on two main facts. First, PRF is a main biological source of many growth factors, cytokines as interleukins, and multiples cells as immune cells, which stimulate and accelerate neo-vasculogenesis in the grafted area and surrounding tissue. So, the stimulating impact of PRF on blood vessels enhanced the growing of new capillaries to perfuse the skin graft. [11-13, 15]. Secondly, poor adherence or seroma formation can disrupt vascularization of graft and lead to graft failure, the application of PRF as fibrin gel between wound bed and the graft acts as adherent substance that led to adheres the entire surface of the graft to the wound bed and minimize contraction and scarring of grafted area [7,9,12, 35]. Identical result for this outcome described by other researchers whom studied the influence of PRF on skin grafts, they showed up in their studies efficiency impact of PRF in the skin graft take and survival and indicated that fibrin gel for PRF capable to improvement and enhancement graft survival and lowering their loss. It can diminish the hematomata and edema formation at the recipient graft site and also improve direct adhesion of graft, which result in raising the blood supply to the graft derived from the recipient place and inhibit shearing [36,37].

Graft survival can determine the success of the skin graft healing as the more the skin color resemble the origin skin the more success of skin graft healing, which indicating a good acceptance. According to all groups graft treated with PRF, it appeared the highest survival rate. This indicated the positive role of PRF in affecting graft survival. The reason for this may be attributed to the positive role of PRF in increasing the blood supply to the graft region. This is due to its content of various growth elements as VEGF which has a major role in accelerating the formation of new blood vessels and thus improving healing. This result is consistent with [1, 23]. Also, the graft survival was more

quickly in the sBMAC group compared to the pb-prf group. Superior graft survival in group sBMAC may be attributed to the higher content of growth factors in bone marrow-derived platelet rich fibrin compared to the type derived from peripheral blood. Where the Shoji et al., showed that the quantity of growth factors as VEGF and FGF in fibrin derived from bone marrow is greater than that of fibrin derived from peripheral blood and the bone marrow fibrin has superior ability for the cell proliferation and differentiation than the another fibrin [38].

The micro-histopathological examination revealed that grafted groups treated with PRF showed speedier grafts healing compared to the control group, which was represented by fewer inflammatory cells, higher angiogenesis, better formation and maturation of the granulation tissue and faster re-epithelization. Other Previous researches like [Tang et al. 2023] have exhibited similar promising results of PRF therapy in hastening and improving skin graft take and wound healing thru delivering PRF to skin graft area [39]. Anti-inflammatory properties of PRF is due to the acting of different leukocytes present in it together with platelets, that lead to increase release level of the anti-inflammatory factors and further change the inflammatory activity towards the tissue regeneration activity. PRF improves wound microenvironment by regulating inflammatory response and supporting neo-vascular formation. Anti-bacterial effect of PRF may be due to existing platelets in PRF that can interact with different bacterial strains and immune cells through their surface receptors. Platelets can form compounds with neutrophils causes release of reactive oxygen species or formation of neutrophil extracellular traps [7,16,34].

The comparison investigating pb-prf versus a sBMAC yielded difference in terms of graft survival and healing but the variance not statistically significant. Superior improvements in skin graft healing were observed in sBMAC group when compared to pb-prf and control group. According to the our investigators sBMAC treatment led to better cell migration, vasculogenesis, cell proliferation, granulation tissue maturation, re-epithelization and higher anti-inflammatory action potential than pb-prf or control group. A study conducted by Koyanagi et al., showed that although the sBMAC clot smaller than pb-prf, it is comprised heavier networks of fibrin contain significantly higher stem cells, platelets, leukocytes and growth factors in comparison to pb-prf. They showed in their study that treatment of osteoblast with sBMAC in vitro led to greater cell migration, angiogenesis, and collagen synthesis, and a higher osteoblast differentiation potential than bm-prf. Hence, it was proposed that sBMAC may well be a new applicant to improve and advance tissue healing and regeneration [17]. Analyzed contents of sBMAC and bp-prf by ELISA test obtained with Shoji et al., and found that the amount of growth factor is statically greater in sBMAC than that of pb-prf [38].

Collection of bone marrow needs skill and is more annoying to the examiner than arterial and venous blood specimen. In the current study, in all dogs the bone marrow harvesting was without any complicated incident, none of the dogs' evolved infection, bleeding or other complications.

Conclusion

Major differences in skin graft survival and healing were seen between the two types of PRF treatment and non-treated animals. On the other hand, sBMAC treatment led to greater efficiency in graft take and healing than pb-prf Therefore, it was suggested that sBMAC was a new biological materials candidate to encourage skin graft survival and regeneration.

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Funding statement

The funding of research was founded by the authors. Conflict of Interest

The authors declare that there is no conflict of interest regarding the current study.

Ethical consideration

Every part of experiment in the dogs was conducted in agreement with the fundamental ethical guidelines. A local ethical committee of the University Mosul, Iraq approved the project after evaluating ethical standards at all stages of executing operations and handling. [UM.VET. 2023.049] was the approval letter.

Authors' contribution

The authors each contributed equally.
Fig. 1. Shows bone marrow aspiration from femur bones

Fig. 2. (A) - Illustrates withdrawal of pb-prf after preparation from the centrifuged tube, (B) - Illustrate withdrawal of sBMAC after preparation from the centrifuged tube.
Fig. 3. (A): shows removal of skin graft from the animal's back. (Band C): fixation of skin graft in the recipient site by simple interrupted knot. (D): insertion method of PRF under the skin graft in group 2 and 3.

TABLE 1. Shows the criteria adopted to determine the skin graft color change score (survival rate score)

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color of skin graft same as the surrounding skin</td>
</tr>
<tr>
<td>2</td>
<td>Color of skin graft appear as hyperemic</td>
</tr>
<tr>
<td>3</td>
<td>Color of skin graft appear as ischemic appearance</td>
</tr>
<tr>
<td>4</td>
<td>Color of skin graft appear as black</td>
</tr>
</tbody>
</table>

TABLE 2. Shows the criteria adopted to determine the skin graft cosmetic appearance

<table>
<thead>
<tr>
<th>Grade</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (excellent)</td>
<td>when the entire area of the graft was filled with healthy skin appearance</td>
</tr>
<tr>
<td>2 (good)</td>
<td>when up to 20% of the graft presented scabs formation</td>
</tr>
<tr>
<td>3 (reasonable)</td>
<td>when 20% to 80% of the graft was replaced by scabs</td>
</tr>
<tr>
<td>4 (poor)</td>
<td>when more than 80% of the graft was replaced by scabs</td>
</tr>
</tbody>
</table>

TABLE 3. Histopathologic scoring system according to Arslantas et al., 2015

<table>
<thead>
<tr>
<th>Criteria</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory cells</td>
<td>None</td>
<td>Scant</td>
<td>Moderate</td>
<td>Abundant</td>
</tr>
<tr>
<td>Amount of granulation tissue</td>
<td>None</td>
<td>Scant</td>
<td>Moderate</td>
<td>Abundant</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>None</td>
<td>Up to 5 vessels/HMF (high-powered magnification field)</td>
<td>6-10 vessels/HMF</td>
<td>&gt;10 vessels/HMF</td>
</tr>
<tr>
<td>Maturation of Granulation tissue</td>
<td>Immature</td>
<td>Mild maturation</td>
<td>Moderate</td>
<td>Full</td>
</tr>
<tr>
<td>Re-epithelialisation</td>
<td>None</td>
<td>Partial</td>
<td>Complete, but immature/thin</td>
<td>Complete and mature</td>
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</tbody>
</table>

TABLE 4. Observation of skin graft survival

<table>
<thead>
<tr>
<th>Observation day</th>
<th>Control</th>
<th>pb-prf</th>
<th>sBMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>3.2± 1.0 *</td>
<td>2.4± 0.9 *</td>
<td>2.3± 0.6 *</td>
</tr>
<tr>
<td>14</td>
<td>2.6± 1.2 b*</td>
<td>2.0± 0.3 *</td>
<td>1.7± 0.0 *</td>
</tr>
<tr>
<td>28</td>
<td>2.0± 0.6 c*</td>
<td>1.3± 1.0 b</td>
<td>1± 1.0 c</td>
</tr>
</tbody>
</table>

Different value in the same column (a-c) indicate significant in the same group and row (*) indicate significant variance between groups at threshold (p<0.05). Mean ±S.E.

Fig. 4. Graph illustrates skin graft takes ratio of all experimental groups. (*): significant at p<0.05.

Fig. 5. Graph shows seroma formation ratio for all groups. (*): significant at p<0.05.

Fig. 6. Graph show graft weeping occurrence. (*): significant at p<0.05.
Fig. 7. Graph show graft survival score, Different value (a-c) indicate significant in the same group and row (*) indicate significant variance between groups at threshold (p<0.05).

Fig. 8. Gross picture for a grafted area in the all groups of experiment at 7th, 14th and 28th days after grafting. Note the partial loss of graft in control group at 7th day. Also at 28th days: At 28th post grafting days, the figure show greater scar tissue formation in control group than in the other groups and show the excellent cosmetic appearance of graft in sBMAC group.
Fig. 9. Histological image of dog skin from the control group (7 days) shows the wound site (↔) with the inflammatory crust (ic), containing highly inflammatory cells infiltration (score 3) (i), fibrinous exudate (F), high granulation tissue (score 3) (GT), hyperkeratosis (K), destruction of epithelium with slight re-epithelialization (score 1) (R). H&E stain, 100X.

Fig. 10. Histological image of dog skin from the control group (14 days) shows the wound site (↔) with the inflammatory exudate (ic), containing inflammatory cells (score 2) (i), fibrinous exudate (F), granulation tissue (score 2) (GT), hyperkeratosis (K), re-epithelialization (score 2) (R), and skin edge (S). H&E stain, 100X.

Fig. 11. Histological image of dog skin from the control group (28 days) shows the wound site (↔) with mild inflammatory cells infiltration (score 1) (i), granulation tissue (score 2) (GT), hyperkeratosis (K), re-epithelialization (score 3) (R), angiogenesis (A) and skin edge (S). H&E stain, 100X.
Fig. 12. Histological image of dog skin from the pb-prf group (7 days) shows the wound site (↔) with the inflammatory crust (ic), containing inflammatory cells (score 2) (i), fibrinous exudate (F), granulation tissue (score 2) (GT), hyperkeratosis (K), re-epithelialization (score 1) (R), and skin edge (S). H&E stain, 100X.

Fig. 13. Histological image of from the pb-prf group (14 days) shows the wound site (↔) without inflammatory cells infiltration (score 0), granulation tissue (score 2) (GT), hair follicle formation (HF), hyperkeratosis (K), complete re-epithelialization (score 3) (R), and skin edge (S). H&E stain, 100X.

Fig. 14. Histological section from the pb-prf group (28 days) shows the wound site without inflammatory cells infiltration (score 0), granulation tissue (score 2) (GT), with high angiogenesis (score 2) (A), complete re-epithelialization with thick epithelium (score 3) (R), formation of hair follicle (HF), and sebaceous gland (SbG). H&E stain, 100X.
Fig. 15. Histological section of dog skin from the sBMAC group (7 days) shows the wound site (↔) without inflammatory cells infiltration (score 0), granulation tissue (score 2) (GT), re-epithelialization (score 2) (R), angiogenesis (score 1) (A) and skin edge (S). H&E stain, 100X.

Fig. 16. Histological section of dog skin from the sBMAC group (14 days) shows the wound site (↔) without inflammatory cells infiltration (score 0), granulation tissue (score 3) (GT), with high angiogenesis (score 2) (A), complete re-epithelialization with thick epithelium (score 3) (R). H&E stain, 100X.

Fig. 17. Histological section of dog skin from the sBMAC group (28 days) shows the wound site without inflammatory cells infiltration (score 0), no granulation tissue (score 0), with complete re-epithelialization with thick epithelium (score 3) (R), and normal architecture as hair follicle (HF), sweat gland (SG), and sebaceous gland (SbG). H&E stain, 40X.
References


تأثير الفيبرين الغني بالصفائح الدموية المشتق من الدم المحيطي و نخاع العظم (مركز شفط نخاع العظم الصليبي) على التئام رقع الجلد الذاتية في الكلاب: دراسة مقارنة

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الخلاصة

الفيبرين الغني بالصفائح الدموية المشتق من الدم المحيطي هو واحد من أكثر مركزات الصفائح الدموية الجديدة والأكثر استخدامًا في المجال العلاجي الطبي وله دور أساسي في إصلاح الأنسجة والشفاء لأنه يعد مصدر لمجموعة واسعة من أنواع مختلفة من عوامل النمو، الجزيئات المضادة للالتهاب وجزيئات ما بعد الالتهاب. من جهة أخرى، تم مؤخرًا استعمال نخاع العظم كمصدر للفيبرين الغني بالصفائح الدموية لتسريع التئام الجروح بدلاً من مصدر الدم المحيطي سواء الشرياني أو الوريدي. تم إجراء هذه التجربة لدراسة تأثير الفيبرين الغني بالصفائح الدموية المشتق من نخاع العظم على تقبل رقع الجلد الذاتية كاملة السماكة، ودمجها، وانتهاجها، ومغطاة تأثيره مع تأثير الفيبرين المشتق من الدم المحيطي. تم دراستها الحالي 27 كلبا. تم فيها ترقيق الجلد بشراع المريض. تم فيها ترقيق الجلد من دون أي علاج طبيعي، مجموعة الفيبرين المشتق من الدم المحيطي: تم فيها إضافة الفيبرين المشتق من الدم المحيطي ومجموعة الفيبرين المشتق من نخاع العظم والتي تم فيها علاج مكان الترقيق بالفيبرين الغني بالصفائح الدموية المشتق من نخاع العظم.

تم فحص مكان الترقيق عيانا خلال 28 يومًا بعد أخر الرفع. تم فحص مكان الترقيق عيانا خلال 7 و 14 و 28 بعد العملية. أظهرت النتائج أن كلا النوعين من الفيبرين لهما تأثير تحفيزي في دمج رقع الجلد مع سرير جرح مكان الترقيق والتنام، والذي تميز بتقبل الفراغ بنسبة 100%، والصورة التنبؤية الممتازة للرقب، تعزيز تكون الأنسجة الحبيبية، وضعها، سرعة إعادة الطهارة وشفاء حيوية الرقب بشكل أفضل على عكس مجموعة السيطرة، التي أظهرت نقص كمي للرقب وتأخر نتائج الأنسجة الحبيبية وعاجة الطهارة والإنتان. إلى جانب ذلك، أظهرت النتائج أن الفيبرين الغني المشتق من الدم المحيطي و نخاع العظم نتيجة التماثل أفضل خلال جميع مراحل عملية شفاء الرقب مقارنة بالفيبرين المشتق من الدم المحيطي. تستنتج من هذا البحث أن الفيبرين الغني بالصفائح الدموية المشتق من نخاع العظم له فاعلية أكبر من الفيبرين الغني المشتق من الدم المحيطي.

الكلمات المفتاحية : الفيبرين الغني بالصفائح الدموية، نخاع العظم، الرقب الجلدية.