Effects of platelet-rich fibrin on skin pedicle graft healing (Clinical and biochemical study)

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Abstract---Objective: Avulsion injuries mostly lead to serious lesions. and those wounds become a challenge to be repaired by surgeons. The aim of the study was to evaluate the effect of autogenic PRF on skin pedicle graft to treat experimental avulsion wounds including clinical and hydroxyproline evaluations. Materials and methods: The experiment was performed on 15 adult female goats, aged between 7-8 months and weighted 18-20 Kg. Surgery was performed under heavy sedation and complete aseptic conditions, 2 skin marks (2×2 cm) were created on both sides of the trunk region of the animal. Two full thickness of the marked skin is cut carefully. A sliding flap (2×6 cm) are harvested parallel to the lines of least tension and are then slid over the adjacent wound which were closed by 2-0 nylon monofilament suture. These wounds are classified into two groups, the first one as the control group and the second group treated with autogenic platelet-rich fibrin. The two groups are represented in each goat. Results: Clinical examination showed the amount of wound exudation in the treated group was a significant increase compared to the control group. The swelling of the grafts in the treated group disappeared within the next three postoperative days. However, in the control group, it was greater and remained for a longer period, as it continued until the seventh day. Biochemically, the amount of hydroxyproline that was present on days 7 and 14 after grafting in the control and treatment groups was considerably more significant than in the normal group. On days 21 post-surgery, the hydroxyproline content showed no significant difference between treated, control and normal groups, but there was slight decrease in control group. Conclusions: the platelet rich fibrin group had the potential for more
rapid return to normal function, and indicate the improvements associated with introducing PRF into the repair skin pedicle graft.

Keywords—avulsion wound, pedicle graft, PRF, Skin, hydroxyproline.

1 Introduction

Avulsion injuries are rare but mostly lead to serious lesions. They are defined as the forceful detachment of the skin and subcutaneous fat layer from underlying tissues. The injury mechanisms are severe, suddenly appearing shearing forces mostly occurring in the context of a motor vehicle or machinery accidents, depending on the severity or nature of the trauma as well as the magnitude and duration of the energy impact (De Coninck et al. 2017).

Pedicle flaps are a type of graft in which a portion of skin and subcutaneous tissues are transferred to preserve a neurovascular pedicle. Unlike flaps, free skin grafts are devoid of all vascular and neural connections and rely on the recipient site for survival. Skin flaps are dependent upon the continuation of adequate circulation until vascularization takes place. Because they are much thicker than grafts, this is a slower process, and the flap is vulnerable to problems of kinking or tension of the base (Browne, 1986).

The continuum of wound repair may be broken into three distinct phases: inflammation, proliferation, and maturation. The elimination of wound pollutants and damaged or dead tissue occurs shortly after the beginning of the inflammatory phase of the healing process, which is characterized by the activation of inflammation inside the injured tissue. The proliferative phase is the phase of tissue healing that reflects the period during which blood flow, components of the extracellular matrix, and the epithelial covering of the wound are all restored. This phase occurs during the restoration of injured tissue. The maturation phase sometimes referred to as the remodeling phase, is characterized by the restructuring of collagen and the restoration of at least part of the pre-wound level of tissue strength. (Gushiken, et al. 2021 ; Teller and White, 2009 and Alfars, 2009).

Platelet-rich fibrin, often known as PRF, is an autologous platelet concentrate of the second generation that is generated without the use of anticoagulants or any other chemicals. Recently, the effects of PRF on tissue-wound healing have been described in several systematic studies, showing that PRF has long-term impacts on the healing process. One of the most significant and well-documented benefits of PRF is the presence of leukocytes, which are cells of the host immunological defense system that actively work to combat infection. In addition, PRF was first created with high centrifugation rates, which enabled a fibrin clot to form. This fibrin clot can potentially be used as a three-dimensional scaffold, further accelerating the healing process. (Wang, et al. 2017).

When studying cutaneous wound healing in animals, it is essential to quantify the creation of new collagen as well as the quality of this collagen. The connective tissue is mainly made up of collagen, the main component. The correct

The aim of the study was to evaluate the effect of autogenic PRF on skin pedicle graft to treat experimental avulsion wounds, the evaluations were performed include clinical and biochemical (hydroxyproline) evaluations.

2 Materials and Methods

The experiment was conducted on 15 adult female goats, aged between 7-8 months and weighted 18-20 Kg. Before surgery, ten milliliters of blood were drawn from the jugular vein. The blood was immediately transferred to a 10-mL sterile plastic tube (PRF Tube; without anticoagulant, evacuated tube), and centrifuged at 1500 rpm for 14 min in a centrifuge (figure 1). The resultant product consists of three layers. The topmost layer consists of acellular PPP (platelet-poor plasma), PRF clot in the middle, and RBCs at the bottom of the test tube (Figure 2). The fibrin clot obtained after centrifugation is removed from the tube and the attached red blood cells scraped off from it and discarded (figure 3). Surgery was performed under heavy sedation and complete aseptic conditions, 2 skin marks (2×2 cm) were created on both sides of the trunk region of the animal. Two full thickness of the marked skin is cut carefully (figure 4, a). Advancement or sliding flap (2×6 cm) are harvested parallel to the lines of least tension and are then slid over the adjacent wound (figure 4, b). These wounds are allocated into 2 groups, the 1st one as the control group (without local treatment) and the second group treated with autogenic platelet-rich fibrin as PRF-treated groups (figure 4, c). The 2 groups are represented in each goat. The edges of the wounds were closed with a simple interrupted pattern by 2-0 nylon monofilament suture. Occlusive dressing was used to protect the grafts from dryness and infection. Clinically wound assessment post-surgery was performed as the following considerations: The general health condition of the animal, Color of the skin wound, Swelling, and exudates.

The exudates scores were estimated as non (1), light (2), moderate (3), and heavy (4), while swelling scores classified into non 0, mild 1, and obvious 3 (Nikhah et al. 2013). The measurement of hydroxyproline allowed for the analysis of collagen and cross-links. As previously mentioned, samples were collected, and their hydroxyproline concentration was measured using a modified spectrophotometer technique. At 7, 14, and 21 days after surgery, collected samples from the control, PRF, and normal skin groups.

The samples were hydrolyzed in 6 molar HCl for 14–16 hours at 105°C, and the hydroxyproline was then oxidized by chloramine. Then, T. Ehrlich’s reagent was used, and incubation at 60°C was required to produce a chromophore. The hydroxyproline product in the alkaline medium was extracted into toluene and subsequently into the acid phase to eliminate interfering chromophores. The hydroxyproline content was determined using a calibration curve based on standard solutions run in the same manner as samples, and the acid phase’s absorbance was measured at 543 nm. In parallel with sampling for
hydroxyproline analysis, 50–100 mg of each sample was put on a plate and dried in an oven at 100°C for three hours to calculate the percentage of dry matter (DM) in each sample. The last step was to express the hydroxyproline content of skin samples in µg/r g DM. [Al-Khalifa, et al. 2019].

3 Results and Discussions

Clinical evaluations

There was no change in the animal's appetite after the surgery. The temperature in all animals increased by one degree on the day of the surgery and returned to normal. On the first postoperative day, fluid exudation was at the site of the wound in the treated group, while in the control group, the exudation in small amount was observed. On the second, third, and fourth post-surgery, it was observed that there were a small number of exudates and that exudation gradually disappeared in the treated wounds. However, there was disappearance of fluid exudates in the control wounds at the 3rd day (figure 5, table 1).

Swelling was observed in the patched skin area on the first and second day in the treated group of wounds and then the swelling disappeared within the next three days. However, in the control group, the swelling was greater and remained for a longer period, as it continued until the seventh day (figure 6, table 2).
Figure 2b: the accumulation of PRF inside tube after centrifugation

Figure 3: the PRF glue was isolated from the blood

Figure 4a: skin pedicle flap was done by 2 parallel incisions

Figure 4b: Suturing the pedicle flap was complete with simple interrupted suture pattern
Figure 6: clinical study showed the exudates scores for skin grafts of Control and PRF treated groups wounds during (1, 2, 3 and 4 days post-surgery). ABC Different letters among groups indicates significant differences (P < 0.05).

Table 1: clinical study showed the exudates scores for skin grafts of Control and PRF treated groups wounds (1, 2, 3 and 4 days post-surgery) (means and standard errors)

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2±0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.9±0.17&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.1±0.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>PRF</td>
<td>3.1±0.17&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.0±0.14&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.9±0.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 4c: the site of surgery was covered by wound dressing

Figure 5: measuring the level of acidity by using PH test paper
ABC Different letters within each column indicates significant differences (P<0.05).

Figure 6: clinical study showed the Swelling scores for skin grafts of Control and PRF treated groups (1-8 days post-surgery). ABC Different letters among groups indicates significant differences (P<0.05).

Table 1: clinical study showed the swelling scores for skin grafts of Control and PRF treated groups (1-8 days post-surgery) (means and standard errors)

<table>
<thead>
<tr>
<th>Group</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
<th>8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.1±0.1A</td>
<td>2.9±0.1A</td>
<td>2.7±0.15A</td>
<td>2.6±0.16A</td>
<td>2.1±0.1A</td>
<td>1.9±0.17A</td>
<td>1.6±0.16A</td>
<td>1.0±0.0A</td>
</tr>
<tr>
<td>PRF</td>
<td>2.8±0.13A</td>
<td>2.2±0.13B</td>
<td>1.8±0.13B</td>
<td>1.2±0.13B</td>
<td>1.0±0.0B</td>
<td>1.0±0.0B</td>
<td>1.0±0.0B</td>
<td>1.0±0.0A</td>
</tr>
</tbody>
</table>

ABC Different letters within each column indicates significant differences (P<0.05).

**Hydroxyproline evaluation**

The results of hydroxyproline content were summarized on table 2 figure 6. The hydroxyproline content was significantly higher on days 7 and 14 after grafting in the control group and treated group than in normal group. On days 21 post-surgery, the hydroxyproline content showed no significant difference between treated, control and normal groups, but there was slight decrease in control group.
Figure 6: Biochemical study showed the hydroxyproline content for normal skin and skin grafts of Control and PRF treated groups during different periods post grafting (7, 14 and 21 days post-surgery). ABC Different letters among groups indicates significant differences (P< 0.05). abc Different letters within group indicates significant differences (P<0.05).

Table 1: study of hydroxyproline content for normal skin and skin grafts of Control and PRF treated groups during different periods post grafting (7, 14 and 21 days post-surgery) (means and standard errors)

<table>
<thead>
<tr>
<th>Groups</th>
<th>7th day</th>
<th>14th day</th>
<th>21th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>144130.4±1014.5Aa</td>
<td>144130.4±1014.5Aa</td>
<td>144130.4±1014.5Aa</td>
</tr>
<tr>
<td>Control</td>
<td>170961.3±3057.9Ba</td>
<td>191101.8±5229.5Bb</td>
<td>131302.2±6481.9Ac</td>
</tr>
<tr>
<td>PRF</td>
<td>160707.6±3286.7Ca</td>
<td>190775.2±2883.7Bb</td>
<td>142514.0±7283.2Aa</td>
</tr>
</tbody>
</table>

ABC Different letters within each column indicates significant differences (P<0.05). abc Different letters within each row indicates significant differences (P<0.05).

Discussion

The importance of dealing with wounds is due to the possibility of infection of the wound due to the large size of the avulsion part from the skin, usually caused by attacks of predators, injuries to industrial equipment, or car accidents (Pavletic, 2018). The aim of the study was clinical and biochemical (hydroxyproline content) evaluation of skin pedicle grafts treated with autogenic PRF. Clinical examination showed the amount of wound exudation in the treated group was in a significant increase compared to the control group. However, these exudates are showed in acute wounds in the normal range of inflammatory stage period. Therefore, these excessive healthy acute exudates induced by PRF promote the healing process in the treated group.

Exudates are sometimes called "wound fluid" and "wound drainage." A rupture in the skin initiates an inflammatory reaction and causes the capillaries to become more permeable. Serous fluid seeps into the wound bed and serves as the
foundation for exudates, water, electrolytes, minerals, proteins, inflammatory mediators, proteases, growth factors, white blood cells, and microbes. It is a natural element of the healing process and provides a moist environment that permits epithelial cells to move over the wound. The growth agents and nutrients included in exudates are essential for wound healing, and the wet climate also promotes autolysis (the separation of necrotic tissue from healthy tissue). (Nichols, 2016).

In the treated group, the swelling disappeared within the next three post operative days. However, in the control group, the swelling was greater and remained for a longer period, as it continued until the seventh day. From a clinical standpoint, this stage is distinguished by symptoms like redness, heat, swelling, and pain when seen from a clinical perspective. These symptoms are the direct consequence of mast cells releasing vasoactive amines and histamine-rich granules into the bloodstream. These mast cell mediators induce the vessels around the injury to become leaky, making it possible for neutrophils to migrate quickly and efficiently from the vasculature to the area of damage. As a result of the veins being leaky, fluid can flow into the region, producing swelling, which in turn causes discomfort due to pressure. (Goldberg and Diegelmann, 2010; Broughton, et. al. 2006 and Cutting, et al 2015). As we knew in the treated group, we added PRF, which is rich with inflammatory cell, growth factors and fibrin, this result in acceleration of the debridement or inflammatory stage and speeding up proliferation stage, this result agreed with (Ding, et al. 2017).

Granulation tissue formation characterizes the period of new tissue creation. Intense fibroblast proliferation and migration are necessary for synthesizing new extracellular matrix in the region of injured tissue. Once in the wounded tissue region, fibroblasts generate new matrix elements such as proteoglycans, glycosaminoglycans, and collagen, which are deposited in the damaged area to replace the original temporary matrix, which was initially composed of fibrin. The primary structural component of granulation tissue is collagen, which strengthens the extracellular matrix. The amino acid proline is an essential component of the collagen fiber, and hydroxyproline is a biochemical marker for collagen tissue that indicates the healing process is progressing. Therefore, the hydroxyproline test is the gold standard for evaluating collagen formation. (Caetano, et al. 2016).

The hydroxyproline content was significantly higher on days 7 and 14 after grafting in the control and treated groups than in normal group. Hydroxyproline is an uncommon amino acid present in the collagen fibers of granulation tissues. Biochemical analysis revealed increased hydroxyproline content, which is a reflection of increased cellular proliferation and therefore increased collagen synthesis, during proliferation stage (Chen, et al. 2012).

On days 21 post-surgery, the hydroxyproline content showed no significant difference between treated, control and normal groups, but there were slight decrease in control group. This result comes from action of Platelet-derived growth factors and TGF- which have been identified in PRF (He, et al. 2009). Platelet derived growth factors increase cell proliferation and migration, and collagen production, and it shown to enhance regeneration of the graft (Bosch, et
In addition, the TGF can promote mechanical strength in healing skin graft by regulating collagen I and III synthesis, cross-link formation, and matrix remodeling (Hou, et al., 2009).

4 Conclusion

1. PRF could promote the regeneration and maturing of the wound and enhance hydroxyproline content in skin pedicle graft.
2. The improvement of biochemical properties for the PRF treated group and relative magnitude of these results compared with those of control group suggest that the PRF group had the potential for more rapid return to normal function, and indicate the improvements associated with introducing PRF into the repair skin pedicle graft.

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References


