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Evaluation of Topical Application of Platelet Gel in Skin Grafted Burn Wounds

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ABSTRACT

Platelet gel (PG) is a hemocomponent obtained by associating activated hyper-concentrated platelets and cryoprecipitate: It allows an exogenous in situ addition of growth factors (GF) with homeostasis restoration and tissue reparation and regeneration. In this study we applied PG topically to split-thickness skin grafted (STSG) burn wounds along with standard treatment to observe the healing process, compared to standard treatment alone without PG. Fifty adult patients of both sex undergoing skin grafts for post-burn raw areas were included in this study. To avoid any bias as well as best standardization of circumstances, raw areas included in the study were symmetrically divided longitudinally in each patient into two halves; in one half, graft was applied with platelets gel (PG graft) while in the other half, graft was applied without the gel (control graft). There was a statistically significant increase in the percentage of healing with platelet gel grafts (0.94±0.08) compared to control grafts (0.89±0.08), t (49)=7.0, $p<0.001$ (two-tailed). In conclusion, this study demonstrates that PG enhances the healing skin grafted post-burn raw areas; so we recommend its use for all cases of raw areas before graft application.

INTRODUCTION

The proper management of burn wounds continues to be a great problem, especially when the wound is deep and extensive. Many attempts have been made to enhance wound healing in partial-thickness wounds but methods are still needed to evaluate the effect of drugs by measuring the rate of healing in skin graft wounds **[1]**.

Injury to the skin results in the physical disruption of the normal cellular architecture and triggers wound healing. Growth factors and cytokines released from inflammatory cells dictate the function of those cells present within the wound. Incorporation of such cytokines and growth factors may be a key factor in the development of new skin replacement therapies **[2]**.

Wound healing is a complex process mediated by interacting molecular signals involving mediators and cellular events; it is followed by mesenchymal cell recruitment, proliferation and extracellular matrix generation which allows scar formation. Wound healing is regulated by a pattern of events including coagulation, inflammation, formation of granulation tissue, epithelialization and tissue remodeling. These events are mediated and modulated by interacting molecular signals, primarily cytokines and growth factors (GF): They stimulate and modulate the main cellular activities which underscore the healing process **[3]**.

Studies on the restoration of tissue integrity have shown the role of the platelets in the wound healing process: During coagulation and the inflammation phase, the formation of a blood clot induces adhesion, aggregation and degranulation of circulating platelets. Platelet α-granules release numerous GF: Platelet derived growth factor (PDGF) **[4]**, transforming growth factor beta (TGF-b) **[5]**, epidermal growth factor (EGF) **[6]**, insulin-like growth factor-1,2 (IGF 1-2) **[7]**, and vascular endothelial growth factor (VEGF) **[8]**. These factors play an important tole in the tissue remodelling phase (re-epithelization and neovascularization) by mesenchymal cell recruitment and extra-cellular matrix synthesis **[9]**.

Technically advanced studies have proved that the use of growth factors on the wound is a successful method of enhancing healing although their final outcome is not yet conclusive. Among these studies, the platelet-derived growth factor (PDGF) is one of the key factors which can stimulate angiogenesis, fibroblast proliferation, collagen synthesis and reverse irradiation impaired wound healing **[10]**.

Platelet gel (PG) is a hemocomponent obtained by associating activated hyper-concentrated platelets and cryoprecipitate: It allows an exogenous in situ addition of GF with homeostasis restoration

with tissue reparation and regeneration. In PG, the GF released from the thrombocyte agranules is induced in "vitro" by adding calcium gluconate, thrombin or batroxobine to platelets. Whole blood, as described in Marx's original technique, is the source for hyper-concentrated platelet and cryoprecipitate: The source may be autologous or homologous, if general or clinical conditions contraindicate an autologous procedure **[10]**.

In burned patients, PG has been used for split-thickness skin graft donor sites expecting for acceleration of epithelization and better scar formation. However, there are few reports on the use of PG for skin-grafted burn wounds. We hypothesize that PG can aid in the healing of skin-grafted burn wounds, as it does for partial skin defects of donor sites **[11]**.

In this study we applied PG topically to split-thickness skin grafted (STSG) burn wounds along with standard treatment to observe the healing process, compared to standard treatment alone without PG.

PATIENTS AND METHODS

Quasi experimental study carried out in the Burn Unit of Suez Canal University Hospital, Isamilia, Egypt, in the period from February 2009 to July 2009. Fifty adult patients of both sex undergoing skin grafts for post-burn raw areas were included in this study this is a. Excluded patients include those less than 12 years of age, small grafts (with surface area less than 5%), grafts that cannot be symmetrically divided and uncontrolled infection of the raw area. To avoid any bias as well as best standardization of circumstances, raw areas included in the study were symmetrically divided longitudinally in each patient into two halves; in one half, graft was applied with platelets gel (PG graft) while in the other half, graft was applied without the gel (control graft).

Study methodology:

All patients were subjected to the same protocol regarding graft harvesting (using Watson knife adjusted to 2nd grade), dressing of grafted area (Vaseline gauze and dressing with tie-over stitches) as well as donor site management. To avoid bias, no excision was done to the wound; just rubbing with dressing prior to graft application. Donor site healing and complications were not included in the present work.

Platelets gel preparation:

At least 48 before the PG application, the platelet concentrate (PC) is kept for 48h at +20ºC with continuous shaking on a horizontal shaker (Forma scientific, Marietta, oH) **[12,13]**. The unit was split into aliquots of 20, 10 and 5ml according to the burn size and is then stored at –40ºC as platelet lysate. Cryoprecipitate is obtained from the cryopreserved fresh frozen plasma by centrifugation at +4ºC, R adjusting the final volume to 40-60ml by adding plasma. The product was split into aliquots of 5ml which were cryopreserved at –40ºC. For platelet gel activation, platelet lysate was thawed at room temperature and moved into a graduated sterile Falcon tube.

Cryoprecipitate, thawed at +37ºC, is added in the Falcon tube with calcium gluconate in the following proportions: 5 parts of platelet lysate, 2 parts of cryoprecipitate, 2 parts of calcium gluconate. The suspension is exposed to slow shaking with the caution to complete 10-12 times a 360º tube revolution, then it is fractionated, according to the burn size and shape, into sterile 10-20ml syringes and left to rest for about 15 minutes. PG was applied as a thin film over the raw area before application of the graft. The grafts were covered by non-adhesive compressive dressing for 5 days, and then dressing was done daily. Graft healing was evaluated at the 10th post-operative day by calculating the percentage of healing related to the original size of each half of the wound separately. The percentage of take, healing time, infection condition, and scar formation were observed.

RESULTS

Fifty burned patients with a mean age of 25.8±13.3 years and ranging from 14 to 60 years were enrolled in the study. Table (1) shows the demographic data of patients. In Table (2) a paired-sample *t*-test was calculated to evaluate the impact of use platelet gel graft on healing degree. There was a statistically significant increase in healing with platelet gel grafts (0.94±0.08) compared to control graft (0.89±0.08), t (49)=7.0, $p<0.001$ (two-tailed). The mean increase in healing was 0.05 with a 95% confidence interval ranging from 0.04 to 0.06. The eta square statistic (0.50) indicated a large effect size.

DISCUSSION

Skin grafting is a simple and common procedure for achieving wound closure. Despite its wide spread use, there is little or no objective information about the outcomes of skin-grafted wounds. When queried, most practitioners would assume that skin-grafted wounds heal quickly. Unfortunately factors that might affect the rate of graft healing have not been well studied **[14]**.

Table (1): Characteristic data of patients (n=50).

Characteristic data	Frequency distribution	
	No.	(%)
Sex:		
Male	32	64.0
Female	18	36.0
Cause of burn:		
Flame	34	68.0
Scald	12	24.0
Electrical	4	8.0
Chemical	0	0.0
Site of burn:		
Upper limb	32	64.0
Lower limb	18	36.0
Trunk	36	72.0
Head & Neck	34	68.0
Surface area of burn:		
Mild <15%	30	60.0
Moderate 15-25%	14	28.0
Major >25%	6	12.0
Swab culture:		
Negative	6	12.0
Positive	44	88.0
Anemia (hemoglobin <10g/dl)	7	14.0
Hypoalbuminemia (<3.5g/dl)	16	32.0

Table (2): Comparing the total percentage of graft take between PG grafts and control grafts (n=50).

Type of graft	Mean (SD)	Mean difference (SD)	95% CI	Sig. test (*p*-value)
Platelet gel grafts	0.94 (0.08)	0.05 (0.05)	(0.04, 0.06)	t (49)=7.0 *p*<0.001*
Control gel grafts	0.89 (0.08)			

* Paired *t*-test (statistically significant at $p<0.05$ and 95% confidence level).

Table (3): Percentage of take with PG grafts compared to control grafts (n=50).

	N	Mean (SD)	F (df)	*p*-value*
Better than control grafts	32	28.7 (14.22)	2.39 (2.47)	0.102
Equal to control grafts	17	21.2 (10.1)		
Less than control grafts	1	12.0		

ANOVA (statistically significant at $p<0.05$ and 95% confidence level).

Table (4): Factors affecting percentage of take with PG grafts.

	Healing with platelet gel graft			
	Better (n=32) No. (%)	Equal (n=17) No. (%)	Less (n=1) No. (%)	Sig. test* (*p*-value)
Sex:				
Male	21 (65.6)	11 (64.7)	0	X^2 (2)=1.82
Female	11 (34.4)	6 (35.3)	1 (100.0)	*p*=0.403
Cause of burn:				
Flame	22 (68.8)	11 (64.7)	1 (100.0)	X^2 (4)=0.939
Scald	8 (25.0)	4 (23.5)	0	*p*=0.919
Electrical	2 (6.2)	2 (11.8)	0	
Surface area of burn:				
Mild <15%	20 (62.5)	10 (58.8)	0	X^2 (4)=8.33
Moderate 15-25%	8 (25.0)	6 (35.3)	0	*p*=0.08
Major >25%	4 (12.5)	1 (5.9)	1 (100.0)	
Site of burn:				
Upper limb	9 (28.1)	8 (47.1)	1 (100.0)	X^2 (6)=4.68
Lower limb	22 (68.8)	9 (52.9)	1 (100.0)	*p*=0.586
Trunk	10 (31.3)	4 (23.5)	0	
Head & Neck	7 (21.9)	8 (47.1)	1 (100.0)	
Anemia (HB <10g/dl)	5 (15.6)	2 (11.8)	0	X^2 (2)=0.304 *p*=0.859
Hypoalbuminemia (<3.5g/dl)	12 (37.5)	4 (23.5)	0	X^2 (2)=1.48 *p*=0.478
Positive swab culture	29 (90.6)	14 (82.4)	1 (100.0)	X^2 (2)=0.859 *p*=0.651

Chi-square test (statistically significant at $p<0.05$ and 95% confidence level).

As burn wound healing involves inflammation, cellular proliferation with formation of fibrovascular and epithelial tissues, growth factors and cytokines released from inflammatory cells dictate the function of those cells present within the wound. Incorporation of such cytokines and growth factors may be a key factor in the enhancement of skin graft healing **[2]**.

The application of growth factors to enhance wound healing with one or multiple applications, topical or parental delivery, is still the subject of wide discussion **[1]**.

Platelet gel is a tool for bringing growth factors to the wound area by a platelet lysate and matrix proteins by cryoprecipitate; it is useful to promote the functional recovery of physiological tissue reparation **[15]**.

Interaction of many growth factors is known to be involved in the wound healing process. PG has been demonstrated to be effective for bone formation in alveolar bone grafts and to produce better scars at split-thickness skin graft donor sites. These observations led us to hypothesize that a mixture of growth factors would affect wound healing **[16]**.

PG, as a source of growth factors, is increasingly used in almost all fields of surgery for the treatment of a variety of soft and hard tissue defects, most notably in accelerating bone formation and in the management of chronic non-healing wounds **[17]**.

In our study we evaluated the role of PG as a source of growth factors in enhancing the healing of skin grafted burn. Fifty adult patients of both sex undergoing skin grafts for post-burn raw areas were included in this study. To avoid any bias as well as best standardization of circumstances, raw areas included in the study were symmetrically divided longitudinally in each patient into two halves; in one half, graft was applied with platelets gel (PG graft) while in the other half, graft was applied without the gel (control graft).

Our study showed that there was a significant improvement of skin graft healing with PRP gel application. On the other hand, healing was not affected by other factors including, age, sex, cause of burn, surface area of burn, site of burn, presence of anaemia or infection.

In our study, only one patient from the study group (PG) had a lower percentage of healing compared to the control graft, a result that might be attributed to a mechanical factor due to excessive shearing movement owing to the position of the raw area in the axilla.

Our results were consistent with the results of the work of Crovetti et al., who showed that in each case granulation tissue formation was increased following the first PG applications, while complete re-epithelization was obtained later. Pain was reduced in every treated patient **[18]**.

Kazacos et al., stated in his study, that the PG is very useful for the management of acute trauma wounds. The mean time needed for healing of wounds treated with PG was 21 days which was significantly lower than the mean time needed for healing of the control group **[19]**.

On the contrary, Vermeulen et al., evaluated healing and deposition of fibronectin in a full thickness wound showed that re-epithelialization and revascularization in the PG group was similar to the saline (control) group. However, qualitative immunofluorescence microscopy for matrix molecules showed more granulation tissue fibronectin in the PG group than in the saline group **[20]**.

PRP gel has been described to be effective in combined soft and bony tissue reconstruction in facial plastic surgery as well in oral and maxillofacial surgery **[21,22,23]**. On the other hand, several authors reported the effectiveness of PG in the treatment of non-healing chronic wounds **[24,25]**. The study of Kazakos K et al., demonstrated the benefits of topical PG in the management of acute soft tissue wounds as patients treated with PRP gel achieved faster healing rates and adequate tissue regeneration in nearly half the time **[19]**.

Preparation of PG is quick, simple, and relatively inexpensive. In addition, it can work with a range of different cell types. All these factors exhibit PG as an ideal biological therapy **[26]**.

In conclusion, this study demonstrates that PG enhances the healing of skin grafted post-burn raw areas. We recommend its use for all cases of raw areas a just before the graft application. Also, these results encourage further clinical study of the method to achieve earlier burn wound healing and to determine with certainty the role of PG for the tissue regeneration in general.

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