Original Article

Platelet growth factors from allogeneic platelet-rich plasma for clinical improvement in split-thickness skin graft

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Abstract:

Background and objectives: Platelets are a source of numerous growth factors which facilitate repair and healing. Thus platelet rich plasma has been increasingly used as a treatment modality in the field of reconstructive surgeries for wound healing. This preliminary study was carried out to explore whether platelet growth factors from platelet rich plasma could be used for enhancement of split thickness skin graft survival. **Materials and Methods:** Twenty patients (13 males and 7 females) requiring split thickness skin graft for various clinical reasons were enrolled in the study. Platelet rich plasma was collected by apheresis and frozen at −80° C. It was thawed at room temperature immediately before its intended application. PRP was applied only on one half of the wound, while another half served as control. Patient was followed for 6 weeks. The effect was assessed at first dressing in terms of graft uptake and subsequently as time taken for complete healing. **Results:** There was 100% uptake of the graft in the area where platelet rich plasma was applied. In the control area, there was complete graft loss in 4 cases, partial loss in 7 cases and complete uptake in 9 cases. **Conclusion:** This study demonstrated promising results on application of PRP to split thickness skin grafts. Further randomized studies with greater sample size may be undertaken to establish platelet rich plasma as a validated treatment modality.

Key words:

Platelet growth factors, platelet-rich plasma, skin graft

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Introduction

There are numerous treatment options and modalities available in the field of wound care. Platelet-rich plasma (PRP) serves as a source of growth factors important in vascularization and regeneration. It is a potential reservoir of essential growth factors, including platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor-beta 1, and insulin-like growth factor which facilitate repair and healing.[1,2] These peptide growth factors are involved in a variety of biologic processes, which help in altering the wound environment to optimize healing conditions.[3] These compounds are released during platelet activation in response to a variety of stimuli, including thrombin, collagen, adenosine diphosphate and even due to membrane disruption by freeze-thaw technique.[4,5] PRP has gained popularity as a treatment modality in the field of orthopedic, oral and maxillofacial, dental, ophthalmological, plastic and reconstructive surgery.[6-12] It has been found to accelerate endothelial, epithelial, and epidermal regeneration, stimulate angiogenesis, enhance collagen synthesis, promote soft tissue healing, decrease dermal scarring, enhance the hemostatic response to injury, and reverse the inhibition of wound healing caused by glucocorticoids.[13,14]

We have hypothesized that the application of PRP in the split-thickness skin graft is a safe strategy to induce positive changes in the wound microenvironment. Hence, this preliminary study was carried out to explore whether platelet growth factors from PRP could be used for enhancement of skin graft survival.

Materials and Methods

The study was carried out in a tertiary care hospital of north India after obtaining approval from Ethical Committee of the Institute. Twenty patients (13 males and 7 females) requiring split-thickness skin graft for various clinical reasons were enrolled in the study. Inclusion was based on the absence of systemic diseases which could hamper the skin graft acceptance such as diabetes mellitus, collagen vascular disease, etc. Written informed consent was obtained from all the patients before initiation of treatment.

Preparation of platelet-rich plasma

Totally, 20 healthy donors, ABO matched with the patient and passing the criteria for plateletpheresis, were randomly included in the study after taking informed consent. Ethylenediaminetetraacetic acid anticoagulated blood sample was collected prior

Table 1: Patient characteristics

Age/sex	Diagnosis	Duration of wound	Wound size and site	Previous treatments	First dressing (1-week)	Result after follow-up
5/male	PBC hand	2 years old injury. Fresh defect after contracture release	8 cm × 4 cm palm (PRP applied to half part)	None	Full take of graft	Complete healing by day 82
12/male	PBC neck and chest	1-year old burn with fresh defect after contracture release	20 cm × 25 cm neck and chest (PRP applied to chest)	None	90% take on chest. No graft uptake on neck	Full take on chest with poor healing on neck. Regrafting done on neck
29/male	Plexiform neurofibromatosis upper limb and shoulder	Since birth progressively increasing	10 cm × 15 cm (PRP applied to half part)	None	Full take	Complete healing by day 110
10/male	PBC neck	2 years old burn	15 cm × 10 cm neck (PRP applied to half part)	None	Full take in applied part minor loss in other	Healed by day 125
30/male	Posttraumatic raw area leg with a compound fracture BB leg	6 weeks old	8 cm × 5 cm (applied) 5 cm × 5 cm (not applied)	Debridement done 4 weeks back	Full take in applied part, partial loss in other	Complete healing by day 92
28/female	PBC hand with epilepsy	2 years old with a fresh wound after contracture release	Syndactyly release of 2nd and 4th webspace done. PRP applied to 4th space	None	Full take in applied part, partial loss in other	Poor healing in nonapplied part
13/female	Scalp AVM	Since birth	20 cm × 15 cm (half applied)	Multiple attempts at excision and recurrence with extensive scalp scarring	Full take in applied part partial loss in other	Complete healing in both parts by day 148
24/male	Scalp AVM	Since 10 years	12 cm × 15 cm (half applied)	None	Full take in both halves	Complete healing in both parts by day 125
7/male	Scalp venous malformation	Birth	10 cm × 10 cm (half applied)	Partial excision with recurrence 1-year back	Full take in applied part, minimal loss in other	Complete healing in both parts by day 80
23/female	PBC neck	4 years	15 cm × 10 cm (half applied)	None	Full take in both halves	Complete healing in both halves by day 136
40/female	Postburn Marjolin's ulcer thigh	10 years	20 cm × 20 cm (half applied)	Excision and primary closure done 1-year back with recurrence	Extensive graft loss in nonapplied part	Poor wound healing in nonapplied part. Required re-grafting
29/female	Fungal granuloma foot postrenal transplant	3 months	8 cm × 8 cm (half applied)	Renal transplant 1-year back	Graft loss in nonapplied half	Poor tissue healing in nonapplied half
27/male	Nonspecific vasculitis bilateral lower limb with gangrene and ischemic ulcer left leg	5 years	10 cm × 10 cm (half applied)	Below knee amputation right leg done 1-year back	Full take in applied part, 80% take in nonapplied half	Complete healing in both halves by day 108
38/male	Carcinoma buccal mucosa and cheek	6 months	Applied over half flap (donor area — forehead)	None	Full take in both halves	Complete healing by day 88
22/male	Scalp AVM	10 years	12 cm × 15 cm (half applied)	Previous failed excision	Graft loss in nonapplied part	Re-grafting done in nonapplied part
35/male	Soft tissue sarcoma abdominal wall	2 years	15 cm × 8 cm (half applied)	Multiple excision with recurrence	Good take in both halves	Complete healing by day 120
29/male	Compound commuted both bone fracture lower limb	6 weeks	10 cm × 15 cm over flap donor site (half applied)	Debridement done 2 weeks back	Full take in both halves	Complete healing by day 154
28/female	Fungal granuloma foot dorsum	6 months	5 cm × 8 cm (half applied)	None	Full take in both halves	Complete healing by day 135
60/female	Recurrent soft tissue sarcoma dorsum foot	6 months	12 cm × 6 cm (half applied)	None	Good take in applied half, partial loss in the other	Complete healing by day 172
25/male	AVM face	3 years	5 cm × 2 cm (half applied)	Glue embolization 48 h previously	Good take in both halves	Healed by day 68

PBC: Postburn contracture; AVM: Arterio-venous malformation; PRP: Platelet-rich plasma

to plateletpheresis procedure, and cell counts were done using automated cell counter (Sysmex KX-21, Cobe, Japan). PRP was collected by apheresis using the discontinuous cell separation method (MCS 3p, Haemonetics, München, Germany). From the final unit, approximately 5 mL of PRP was transferred in satellite tubing attached with main collection bag with the help of sterile connecting device (Composeal, Fresenius, Germany) for sampling. The sample was frozen at 80°C. The frozen segment was shipped to plastic surgery operation theater in a dedicated cool box to avoid any preapplication decay of growth factors. It was thawed at room temperature immediately before its intended application on the wound. The process of freezing, followed by thawing disrupted the platelet cell membrane and facilitated the release of growth factors.

Platelet-rich plasma was applied only on one half of the wound while another half served as control. After placing split-thickness graft, pressure bandaging was done as per routine practice. The patient under study was prescribed medicines and followed-up weekly for 6 weeks. The healing parameters such as wound size, edges, base and surrounding edema were compared to the control half. The effect was assessed at first dressing in terms of graft uptake and subsequently as time taken for complete healing.

Results

Platelet concentration of PRP ranged from 1.15 to 1.23 × 10^9/μL (mean 1.17 × 10^9/μL). The age range of patients was from 5 years to 60 years. There were 6 cases of postburn contractures, 5 cases of benign ulcers, 4 cases of arterio-venous malformations and 5 cases of the other miscellaneous diagnosis. Patient characteristics are summarized in Table 1.

Platelet-rich plasma was applied to one-half of the wound, and the other half was taken as control. There was 100% uptake of the graft in the area where PRP was applied. In the control area, there was complete graft loss in 4 cases, partial loss in 7 cases and complete uptake in 9 cases [Table 1].

Discussion

The clinical use of PRP for a wide variety of applications has been reported in reconstructive surgery. This study was undertaken to assess the efficacy of PRP in terms of graft survival in recipients of split-thickness skin graft and showed encouraging results. Numerous studies from the literature provide strong evidence to support its clinical use. Schade and Roukis found that addition of PRP to split-thickness skin graft recipient sites enhance primary healing and reduce healing time, likely as a result of shearing force reduction and enhancement of the wound environment with growth factors.[15] Adly and Ahmad applied platelet gel topically to split-thickness skin grafted burn wounds along with standard treatment to observe the healing process and found that platelet gel enhances the healing skin grafted postburn raw areas.[16] A study by Kakudo *et al.* revealed that PRP promotes epithelialization and angiogenesis of split-thickness skin graft donor sites. They found that epithelialization progressed more rapidly; pain was milder. Furthermore, the epidermal thickness and number of newly formed vessels in the dermis were significantly greater on the PRP-treated side.[17] In a recent study, Wanden-Berghe *et al.* found clinically a clear improvement in chronic wounds with an accelerated healing on application of PRP activated by calcium chloride.[18]

In the majority of clinical applications, autologous platelets have been used for the PRP formation, which renders it prone to variability. In our study, we have used allogeneic platelets for preparation of PRP as these are available in larger quantities, are safe and affordable, highly standardized in terms of platelet, residual leukocyte and red blood cell content, regulated for centrifugal forces used for their isolation, the temperature of the centrifugation, techniques of separation and processing and the composition of the preservative solution. A study done by Zhang *et al.* has demonstrated the promising use of allogeneic PRP for bone defect treatment with negligible immunogenicity, great healing efficacy, potentially more consistent quality, and no additional health burden to patients.[19]

In our study, we have also observed that in 9 cases, there was full graft uptake and healing on the control area similar to that of the area where PRP was applied. The plausible reason for this was close proximity of both areas which led to percolation of growth factors onto the control area. Growth factors transduce signals through wound macrophages and thus trigger the induction of positive autocrine feedback loops.[20] This may partially enhance the cascade of tissue repair processes required for wound healing in the adjacent nontreated part.

This study demonstrated promising results on application of PRP to split-thickness skin grafts. Our findings could have been better substantiated if we had done the histopathological examination of our cases to reveal epithelialization and angiogenesis of split-thickness skin graft. Further, randomized studies with greater sample size need to be undertaken in this area to establish PRP as a validated treatment modality.

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