E-ISSN: 2616-3470
P-ISSN: 2616-3462

www.surgeryscience.com
2019; 3(4): 373-376
Received: 01-08-2019
Accepted: 03-09-2019

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Effect of autologous bone marrow application in deep burn management: A quasi experimental study

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DOI: https://doi.org/10.33545/surgery.2019.v3.i4g.269

Abstract
Aim: To study effect and efficacy of bone marrow therapy in burn wound healing.
Material and Methods: Two groups of 25 patients each were selected randomly. The patents under study group were treated by debridement followed by autologous bone marrow therapy to wound site whereas patients under control group underwent debridement only. The patients were then followed postoperatively.
Results: Post operatively the patients in study group experienced less pain. Biopsy from wound bed showed average improved fibroblast, macrophage, neovascularisation, and less necrosis per HPF in study group in comparison with that of control group. Seropurulent discharge in control group was observed in 72% cases but in study group it was 28%. Contractures in case group were seen in 32% cases but in control group it was 64%.
Conclusion: Bone marrow cell therapy is a novel method in deep burn wound management in enhancing wound healing and minimizing complication.

Keywords: Deep burn wound, autologous bone marrow, mesenchymal stem cells, granulation tissue

Introduction
Burns constitute an important public health concern in worldwide, accounting about 180000 deaths worldwide. Majority of which occur in low and middle income countries almost two-thirds in African and south east Asian countries. In India, approximately 1000000 people are moderately or severely burnt every year [1].
When thermal injury is extensive and involves >30% TBSA, it risks to life is very high and often recovery is not uneventful. A burn patient's susceptibility to infection is multifactorial. Eschar provides a protein-rich avascular medium for bacterial growth, and the colonization of a burn wound may occur within the first 48 hours after injury. Topical antibiotics and early excision of this eschar decrease, but do not eliminate, bacterial growth; outcome ultimately relies on the rapid restoration of the barrier function of the skin.5–8 Furthermore, the massive cytokine release that accompanies burn injury greater than 20% TBSA impairs T-cell and neutrophils activity, crippling the host defences against bacterial invasion. This immune dysfunction, coupled with deficits in end-organ perfusion, predisposes patients to sepsis and multiorgan failure [2].
Severe burn wound is characterised by the destruction of skin structures, functions and more importantly the loss of the progenitor cell populations that are essential for regenerating and restoring the structures and functions [2]. Until now, autologous skin grafting remains a standard practice in treating severe burns. However, its effectiveness is often challenged in treating severe burn patients with limited donor sites for skin graft harvesting. To overcome the autograft shortage, a variety of alternatives for autologous skin grafts including allogeneic skin, xenografts and synthetic skin substitutes have been widely adopted in burn wound care [3, 4]. While those alternative devices provide temporary wound coverage, they could never replace the skin autograft delivering the essential autologous progenitor cells that could replicate to regenerate skin tissues for permanent wound closure. In past decades, cell-based therapies have emerged as popular choices in conjunction with standard skin grafting techniques for burn wound healing and regeneration of skin structure and functions. Stem cells may offer potential therapies in two broad areas of burns surgery: in the enhancement of burn wound healing and closure and in the attenuation of the immunosuppressant effects of the inflammatory response.
BONE MARROW THERAPY is a novel concept to regenerate the epidermis from the existing

dermal elements from depth of burn by effect of mesenchymal stem cells.

In this study we have applied autologous bone marrow to deep burn site to find its effect in early burn wound healing and epithelisation.

Materials and Methods

Ethical clearance and informed consent of the patients were taken before the study. Fifty deep thermal burn injury patients admitted in one surgical unit during the year 2017-19 at VSS Institute of Medical Sciences and Research Institute, Burla, Odisha, India were randomly selected for study. Out of 50 patients, 25 patients were kept in study group and rest 25 in control group. Debridement and primary wound care were performed in all patients. In the study group, bone marrow was aspirated from the sternum or from iliac crest of the patients using bone marrow aspiration needle under local anaesthesia. Then the aspirate was kept aside after priming with heparin in a sterile syringe. Bone marrow aspirate was then infiltrated into burn wound and the wound were covered with saline soaked gauge. Post operatively the patients were followed and parameters like wound pain, sero-sanguinous discharge, granulation tissue quality from wound on day5 and post operative contractures were studied (Figure 1, 2, 3, 4, 5 and6).

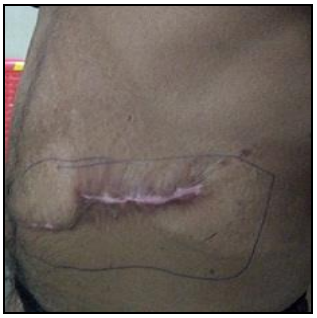

Fig 1: Post operative wound of study on 6th week

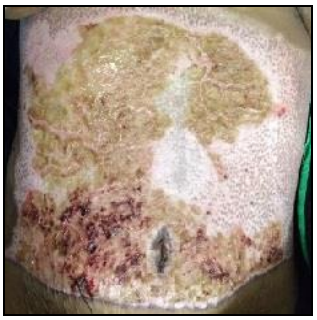

Fig 2: Post operative wound of control on 6th week

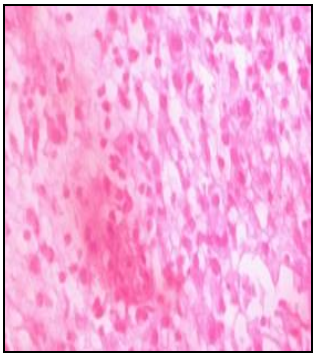

Fig 3: Granulation tissue of study on 5th day

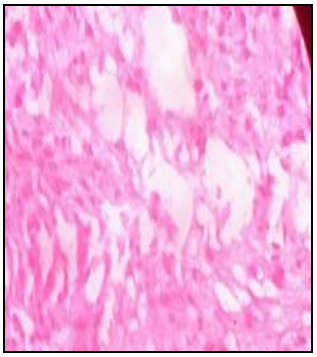

Fig 4: Granulation tissue of control on 5th day

Results

Out of 50 patients with deep thermal burn wounds, 25 patients were non selectively randomised into study group and 25 patients into control group. Post operatively the patients in study group experienced less pain as compared to control group. In the study group patients did not required analgesic after post operative day 7, where as in control group the patient required analgesic until post operative day 16 for wound pain.

Out of 25 patients in control group, contractures were seen in 16 pt but in study group, it was seen in 8 pt out of 25 (P = 0.024). Seropurulent discharge on day5, were seen in 72% of patients in control group where as it was 28% among study group (p = 0.02). Biopsy was taken from floor of wound on post operative day 5 and histopathological examination done; average no. of fibroblast per HPF in study group was 12.2, but in control group it was 3.04. Similarly, macrophage per HPF was 16.96 in study group but it was 5.44 in control group; Neovascularisation and necrosis in both study group were 17.2 & 0.72 respectively, however in control group it were 5.08 & 6.64 respectively [13-15] (Table 1, 2,3 and 4, Figure 7).

Table 1: Post op seropurulent discharge from both study and control group

	Post operative seropurulent discharge on 5th day	
	Yes	**No**
Case	7/25	18/25
Control	18/25	7/25

Table 2: Contracture in both study and control group

	Post operative contracture	
	Yes	**No**
Case	8/25	17/25
Control	16/25	9/25

Table 3: Granulation tissue quality in both study and control group

	Fibroblast/ hpf	**Macrophage/ hpf**	**Neovascularisation/ hpf**	**Necrosis / hpf**
Case	12.2	16.96	17.2	0.72
Control	3.04	5.44	5.08	6.64

Table 4: Showing number of patient who required analgesic in respective post operative day of both study and control group

Post operative day	No. Of patient in control group (required analgesic)	No. Of patient in study group (required analgesic)
1st	25	25
3rd	25	25
5th	25	22
7th	25	13
8th	25	0
10th	21	0
12th	17	0
14th	9	0

Discussion

Mesenchymal stem cells (MSCs) were initially isolated from bone marrow but are now shown to reside in almost every type of connective tissue [4]. MSCs are characterized as a heterogeneous population of cells that proliferate *in vitro* as plastic adherent cells able to develop as fibroblast colony forming-units [5]. MSCs are distinguished from hematopoietic cells by being negative for the cell surface markers CD11b, CD14, CD34, CD45 and human leukocyte antigen (HLA)-DR but expressing CD73, CD90 and CD105. Importantly, the capacity to differentiate into multiple mesenchymal lineages including bone, fat and cartilage is used as a functional criterion to define MSCs [6]. MSCs are clearly capable of responding and modulating their function when exposed to the cells and biochemical factors that are characteristic of an injury environment. Human MSCs migrate preferentially to regions of inflammation [7] and express several chemokine receptors that are necessary to coordinate their homing ability [8]. Furthermore, MSCs have demonstrated chemotaxis toward a variety of wound healing cytokines *in vitro*, including platelet-derived growth factor, insulin-like growth factor-1, IL-8 and TNFα [9, 10].

Concerning the physiology by which stem cells enhance the process of burn wound healing, several studies have been reported. Mansilla *et al*. [11] found role of stem cells in promoting wound healing in burns. In a similar study, Fox *et al*. [12] reported increased levels of bone marrow derived endothelial progenitor cells in burn patients. Focusing on the role of cytokines in burn wound healing, Payne *et al*. [13] used amnion derived multipotent progenitor cells to harvest cytokines and apply them in burn wound healing. The results were improved epithelialisation of the burn. Stem cells have also shown to decrease dermal fibrosis development in burn wound healing in mice [14]. Additionally, stem cell treatment of the skin leads to decreased markers of myofibroblasts and down regulated type I collagen, leading to a decrease in the fibrosis.

Research suggests that stem cells modulate the cytokine flow and may curb the systemic inflammatory response syndrome that accompanies thermal injury. *In vivo*, MSCs attenuate

Proinflammatory cytokine release and nitric oxide production while concurrently up regulating the anti-inflammatory cytokines IL-10 and IL-12 [15]. Infusion of bone marrow-derived stem cells during lipopolysaccharide-induced systemic inflammatory response syndrome in mice delivers an anti-inflammatory effect: levels of the proinflammatory cytokines IL-1, IL-6, and macrophage inflammatory proteins-1_ decline, but concentrations of IL-10 and IL-12 are maintained. The clinical manifestations of these changes merit further investigation, but in theory, infusions of stem cells may attenuate the post burn inflammatory response (figure 5).

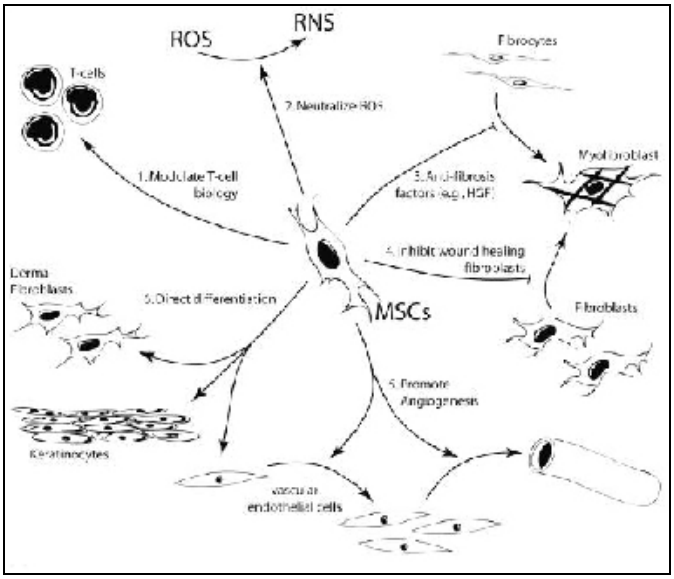

Fig 5: Mesenchymal stem cells can Influence cutaneous regeneration by multiple distinct mechanisms acting on multiple cell types [16]

In our study, autologous bone marrow therapy had facilitated better burn wound healing. This was proved by histopathological examination from wound bed at post operatively Day 5 Neovascularisation per High power field was 17.2 in study group but in control group 5.08; (with P value <0.05) suggest mesenchymal stem cell role in neovascularisation.

HPE from ulcer bed at post operatively day 5 suggest macrophage per HPF in case 16.96 but in control group 5.44 (P value 0.006 (<0.05) suggest recruitment of inflammatory cell that release cytokine IL-4,6 growth factor like fibroblastic growth factor (FGF), epithelial growth factor (EGF) that enhance fibrosis & healing it is prove by HPF study. In study group, average fibroblast per HPF was 12.2 but in control it was 3.04 per HPF suggest role of mesenchymal stem cell (P<0.05). Also necrosis, study group it was 0.72 but in control group it was 6.64 per HPF (P<0.05). Regarding post operatively seropurulent discharge on day 5, 7 among 25 cases in study but in control group 18 among 25 patient were seen (P<0.05). In study group patients did not required analgesics after post operatively day7 where as in control group the patients required analgesic unto postoperatively day 16 for wound pain.

MSC can provide significant benefit during dermal wound healing as they can:

- Accelerate the rate of wound closure and re-epithelisation
- Improve the quality and strength of regenerated tissue
- Recover wound healing pathologies that might otherwise result in a chronic, non-healing wound, and
- Minimize the visual appearance of scar tissue

This study is purely clinical and we have only seen the effects of autologous bone marrow therapy and the rationale behind them are still being studied at molecular level. At present our sample size is small and we will continue our research in more number of patients in future.

References

1. WHO fact sheet on burns providing key facts and information on country data, economic impact, who is at risk, prevention, first aid, WHO response. http://www.who.int/news.room/fact sheets/detail/burns.
2. Kathryn L, Butler, Jeremy Goverman, Harry Ma, Alan Fischman, Yong-Ming Yu *et al*. Stem Cells and Burns:

Review and Therapeutic Implications. J Burn Care Res. 2010; 31:874–881.
3. Amon Francis, Laura Kearney, James Clover. The effects of stem cells on burn wounds: a review. International journal of Burns and Trauma. 2019; 9(1):1-12.
4. Da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006; 119:2204-13.
5. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284:143-7.
6. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8:315-7.
7. Ponte AL, Marais E, Gallay N, Langonne A, Delorme B *et al*. The *in vitro* migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells. 2007; 25:1737-45.
8. Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, *et al*. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by trans differentiation into multiple skin cell type. J Immunol. 2008; 180:2581-2587.
9. Mishima Y, Lotz M. Chemotaxis of human articular chondrocytes and mesenchymal stem cells. J Orthop Res. 2008; 26:1407-12.
10. Hemeda H, Jakob M, Ludwig AK, Giebel B, Lang S *et al*. Interferon gamma and tumour necrosis factor-alpha differentially affect cytokine expression and migration properties of mesenchymal stem cells. Stem Cells Dev. 2010; 19:693-706.
11. E Mansilla, GH Mar´ın, H Drago, *et al*. Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. Transplantation Proceedings. 2006; 38(3):967-969.
12. A Fox, J Smythe, N Fisher *et al*. Mobilization of endothelial progenitor cells into the circulation in burned patients. British Journal of Surgery. 2008; 95(2):244–251.
13. WG Payne, TL Wachtel, CA Smith, MG Uberti, F Ko, MC Robson. Effect of amnion-derived cellular cytokine solution on healing of experimental partial-thickness burns. World Journal of Surgery. 2010; 34(7):1663-1668.
14. Y Wu, S Huang, J Enhe *et al*. Bone marrow-derived mesenchymal stem cell attenuates skin fibrosis development in mice. International Wound Journal. 2014; 11(6):701–710.
15. C Hu, X Yong, C Li, *et al*. CXCL12/CXCR4 axis promotes mesenchymal stem cell mobilization to burn wounds and contributes to wound repair. Journal of Surgical Research. 2013; 183(1):427–434, 2013.
16. Jackson. Stem Cell Research & Therapy. 2012; 3:20.