

Original Article

Role of cord blood and bone marrow mesenchymal stem cells in recent deep burn: a case-control prospective study

Wael Abo-Elkheir¹, Fawzy Hamza³, Ahmed M Elmofty³, Atef Emam², Magdy Abdl-Moktader³, Sameh Elsherefy², Hala Gabr⁴

¹Department Of Immunology, Military Medical Academy, Cairo, Egypt; ²Department of Plastic & Reconstructive Surgery, Medical Military Academy, Cairo, Egypt; ³Department of Plastic & Reconstructive Surgery, School of Medicine, Al-Azhar University, Cairo, Egypt; ⁴Department of Clinical Pathology, Cairo University, Cairo, Egypt

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Abstract: Rationale: Burn injuries represent one of the major worldwide public health problems causing more severe physiological stress than other traumas. Effective treatment of burn injuries is mandatory to prevent the numerous life-threatening complications and possible disabilities. Stem cells, a population of multipotent cells retaining the properties of self-renewal and differentiation, are the main player in tissue regeneration after major trauma. Thus, they are thought to play a key role in wound healing inducing efficient and physiological skin regeneration. Stem cell-based regeneration is quickly gaining scientific grounds. Objective: This study was designed as a comparative prospective study to evaluate and compare the regenerative effect of bone marrow derived mesenchymal stem cells (BM-MSCs) and umbilical cord blood derived mesenchymal stem cells (UC-MSCs) compared to conventional early excision and graft (EE&G) in recent thermal full thickness burned patients. Subject & Methods: Recruited burned patients were randomly divided into three groups (20 patients on each group) having recent thermal full thickness percentage ranging from 10% to 25% total body surface area (TBSA). After receiving allocated treatment, they were assessed as regards: rate of burn healing, presence of post-burn complications both early (such as loss of graft and infections) and late (as hypertrophic scars, keloid, hypo- or hyperpigmentation or contracture of the wound), hospitalization time and cost. Results: This study showed significantly improved rate of healing in both BM-MSC and UC-MSC groups as compared to EE&G group with no significant difference between bone marrow and umbilical cord groups. Comparing the incidence of early complications, partial and total loss of graft occurred in 50% patients in (EE&G) group, while infection complication appeared in 25% of patients of (BM-MSCs) group and in 70% of patients in (UC-MSCS) group. The late complications (hypertrophic scars) were observed in 40% of (EE&G) patients group, in 15% of (BM-MSCs) treated patients group and 20% of (UC-MSCS) patients group. Contractured scars were present in 15% in (EE&G) group, 10% in (BM-MSCs) group, 10% in (UC-MSCS) group. Hypopigmentation occurred in 20% of patients in (EE&G) group, 20% in (BM-MSCs) group and 10% in (UC-MSCS) group. Hyperpigmentation was present in 20% of patient in (EE&G) group, 30% in (UC-MSCS) group but no hyperpigmentation occurred in (BM-MSCs) group. There was no late complication in 5% of patient in (EE&G) group, 55% in (BM-MSCs) group and 30% in (UC-MSCS) group. The results of this study revealed that the hospitalization period was significantly reduced in both (BM-MSCs) group and (UC-MSCS) group as compared to (EE&G) group. Conclusion: this study proves that mesenchymal stem cells, both from bone marrow and cord blood origin, can effectively improve healing of burn injuries.

Keywords: Stem cells, burn, contractured scars, hypopigmentation, graft loss.

Introduction

Burn injury represents one of the important causes of morbidity and mortality all over the world. Proper wound healing requires a complex process of cell migration and proliferation

in addition to the extracellular matrix deposition and remodelling. The standard treatment of choice for burn wounds is autologous skin grafting with subsequent elastic bandage. The major drawback for this option is the limited donor sites in cases of major burns. Proper skin regen-

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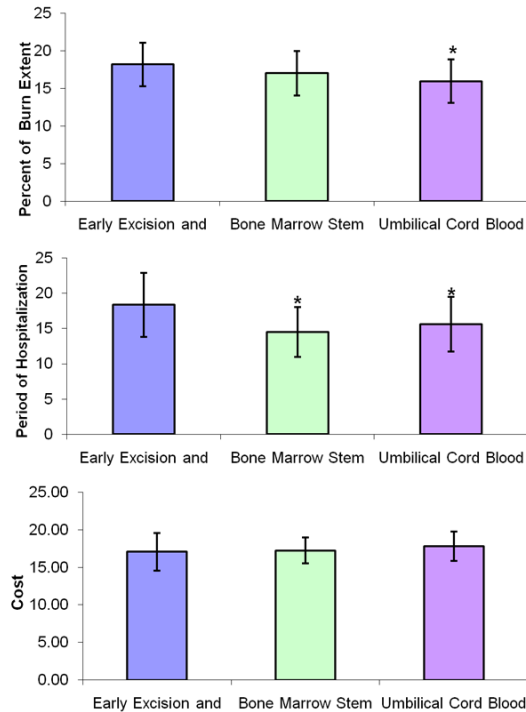


Figure 1. Changes in the percent of burn extent, hospitalization time and the cost of hospitalization in the different studied group. *: Significance by LSD by ANOVA at $P < 0.05$ from early excision and graft group.

eration requires the formation of epidermal appendages destroyed in deep burns such as sebaceous and sweat glands and hair. Bio-engineered skin equivalents do not replace these lost structures. Restoration of such skin appendages requires stem cell-based regeneration. Dermal stem cells have not been successfully cultivated. Hair follicle stem cells represent a possible source of multipotent stem cells in skin; repopulating epidermis, epidermis, hair follicles, and sebaceous glands [1, 2]. However, the main drawback is their scanty number and difficult ex-vivo expansion.

Stem cell application as a treatment option in burn wounds represents a number of repair pathways through both tissue regeneration and prevention of fluid loss and bacterial [3].

Mesenchymal stromal cells (MSCs) represent a population of multipotent adult stem cells abundant in all human tissues. MSCs can be isolated from nearly all body tissues; however, the most common sites are bone marrow, umbilical cord blood and tissues and adipose

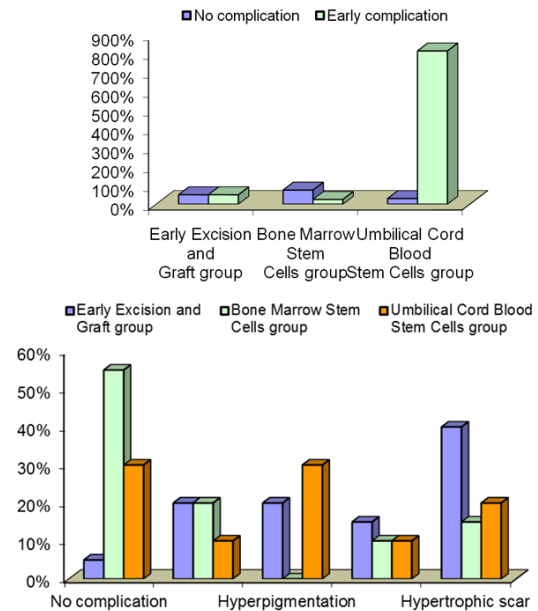


Figure 2. Changes in the frequency of early and late complications in the different studied group.

tissue. MSCs have considerable ability for proliferation in vitro and differentiation into many cell types [4, 5]. Bone marrow derived MSCs are reported to play an important role in accelerating wound healing [6] as well improving the quality of healing through reduction of scar formation and regeneration of normal functioning skin and appendages [3].

Bone marrow derived MSCs have been successfully induced to trans-differentiate into a number of cell lineages including skin [7, 8].

Umbilical cord blood and tissue contains a population of MSCs with the ability to differentiate into bone, skin, endothelium, neural and cartilage lineages [9-11].

Using stem cells for skin regeneration in burns offers a number of advantages. It represents an efficient, high-quality approach for skin coverage especially in deep burns through regeneration of skin appendages in addition to the minimal risk of hypertrophic scarring. In addition, stem cell-mediated skin regeneration lowers morbidity through addressing systemic effects of burn injury, namely hypermetabolism and inflammation.

Stem cell application for burn wound treatment could be performed in the operating room, immediately after debridement. Cells can be

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Figure 3. Photos of patients with early excision and graft.

used as cellular suspension sprayed into the wound with fibrin sealant followed by application of cover in the form of dermal substitute, skin graft or film, to enhance cell paracrine signaling and homing of the cells, improving wound healing [12]. Cells can also be used after seeding on a scaffold or a skin graft.

In addition to their function in wound repair, MSCs also enhance wound healing through for their mostly paracrine anti-inflammatory properties, which can be to modulate severe burns. Current applications of MSCs' anti-inflammatory properties in acute lung injury/respiratory distress syndrome, shows promising results [13].

This study was designed as a case-control prospective study to compare the effect of (BMSCs) and (UCMSCs) with standard regimen in the form of early excision and grafting (EE&G) in the treatment of recent thermal full thickness burned patients with percentage ranging from 10% to 25% total body surface area (TBSA).

Patients and methods

This prospective comparative study was conducted in the Plastic, Reconstructive and Burn Surgery Department of Al-Azhar University, El-Helmia Military Burn Center and Ghamra Military Hospital (Obstetric Department).

Institutional Ethical committee approval was taken before starting the study. All patients signed informed consent.

The study included 60 patients with recent thermal full thickness burned patients with percentage ranging from 10% to 25% total body surface area (TBSA).

Inclusion criteria

Both sexes; Age range: from 15 to 50 years; Patients with recent thermal full thickness burns from 10% to 25% TBSA.

Exclusion criteria

Patients with co-morbidity (chronic diseases as heart diseases, liver or renal impairment, diabetes mellitus and obesity); Below the age of 15 years old and over 50 years old; Patients had inhalation injury; Patients with superficial burns and old burns (greater than two weeks); Patients with other types of burn as chemical, electric and radiation burns; Patients with associated injury in addition to burns.

All patients were fully evaluated in Emergency Department of El Helmia Military Burn Center

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Figure 4. Photos of patients with early excision and graft.

and Plastic and Burn Department in Al-Azhar University.

Then, patients were randomly divided into the following equal groups (20 patients in each group).

Group I (control group): early excision and graft-treated group (EE&G)

They were managed with daily dressing with gentamycin ointment for three to five days to stabilize the general condition of patient, then early excision and graft was performed.

Group II: bone marrow derived mesenchymal stem cells-treated group (BM-MSC)

They were treated by injection of adult stem cells derived from bone marrow. Two days after surgical excision of deep burned tissue, autologous BM-MSCs were injected in the burned area (1 ml/cm² in the concentration of 100,000 MSC/ml). The injection session was repeated after 10 days. Closed dressing with gentamycin ointment was used every three days.

Group III: umbilical cord blood mesenchymal stem cells-treated group (UCMSC)

They were treated by topical umbilical cord-derived mesenchymal stem cells on burned

area. Two days after surgical excision of deep burned tissues, UC MSCs were injected in the same manner as in Group II. Closed dressing with gentamycin ointment was used every three days.

Methods

Complete History Taking: including type of burn, onset, causes, mechanism and site of burn, special habits of medical importance, previous operations and chronic diseases.

General examination: weight of patients, vital signs (pulse, blood pressure, temperature and respiratory rate), chest examination, abdominal examination and other trauma.

Local examination: examination of the burns including surface area (percentage) by using Lund and Browder chart, depth and areas of body affected.

Investigations: routine laboratory investigations were performed including complete blood count, renal function tests, liver function tests, blood gases and serum electrolytes assay at enrollment and every 3 days to assess the general condition of the patients.

Cases were photographed before the onset of treatment, every 2 weeks from the start of treatment and when the burns healed. Post discharging, they were photographed in the follow up at 2 weeks, one month and six months.

Group I (control group)

Early excision and autograft were performed to 20 patients by the following steps on five to seven days after injury: anesthesia: general or spinal; operation: by tangential excision of burned skin with Watson knife. Split thickness skin graft was harvested from healthy skin from either the thigh or the abdomen; The graft was meshed to be porous to increase its surface area to around 1:2 or 1:3 to minimize the area of donor site needed and to allow the drainage of seroma under the graft;

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Table 1. Changes in ages of patients, the extent of burn, percent of burn extent (%), hospitalization time and the cost of hospitalization in the different studied groups

		EE&G	BMSCs	UCBSCS
Ages of patients	Mean ± SD	25.3 ± 4.38	23 ± 1.9	22.9 ± 2.73
	Range	18-35	20-27	18-29
The extent of burn	Mean ± SD	18.15 ± 2.87	17 ± 2.94	15.95 ± 2.89
	Range	15-25	12-22	10-20
Percent of Burn Extent (%)	Mean ± SD	18.15	17	15.95
	P1		NS	< 0.05
	P2			NS
Hospitalization Time	Mean ± SD	18.35 ± 4.53	14.50 ± 3.5	15.6 ± 3.86
	Range	10-25	10-20	10-22
	P1		< 0.005	< 0.05
	P2			NS
The Cost Of Hospitalization	Mean ± SD	17.08 ± 2.49	17.25 ± 1.75	17.80 ± 1.93
	Range	11-20.5	15-20	15-21
	P1		NS	NS
	P2			NS

P1: Significance by LSD by ANOVA at P < 0.05 from early excision and graft group. P2: Significance by LSD by ANOVA at P < 0.05 from bone marrow stem cells group.

Table 2. Description of early complications in early excision and graft group

Early Complications	Frequency	Percent %	Valid Percent	Cumulative Percent
No complication	10	50	50	50
Infection	5	25	25	75
Partial Loss of graft	2	10	10	85
Infection & Partial Loss of graft	3	15	15	100
Total	20	100	100	

Table 3. Description of early complications in BM-MSc group

Early Complications	Frequency	Percent %	Valid Percent	Cumulative Percent
No complication	15	75	75	75
Infection	5	25	25	100
Total	20	100	100	

Table 4. Description of early complications in UCMSC group

Early Complications	Frequency	Percent %	Valid Percent	Cumulative Percent
No complication	6	30	30	30
Infection	14	70	70	100
Total	20	100	100	

the skin graft was fixed using stapler; Dressing with Vaseline gauze for three or five days **Figures 3, 4.**

Groups II and III (mesenchymal stem cells groups)

Anesthesia: general or spinal; Excision of burned skin by tangential excision with Watson knife after three or five days from injury; Group II: BM-MSCs were injected in two sessions: the first was 2 days after surgical excision of deep burned tissue. Administration of already prepared MSCs was done by its injection in the bed of the 20 cm² burned areas (1 mL stem cells in 1 cm² of burn). The second session was done after 10 days. Closed dressing with Gentamycin ointment was used every three days; Group

III: 2 days after surgical excision of deep burned tissues, UC-SC administration was applied topically with gentamycin ointment coverage. The

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Table 5. Comparison of early complications in the different studied groups

	Early Excision and Graft group		Bone Marrow Stem Cells group		Umbilical Cord Blood Stem Cells group		Chi-square test	
	No.	%	No.	%	No.	%	X ²	P-value
No complication	10	50%	15	75%	6	30%	8.142	0.017
Early complication	10	50 %	5	25%	14	70%		



Preoperative photos



7 days post-injection photos



14 days post-injection photos

Figure 5. Photos of patients in the BM-MSC group.

dressing was changed every five to seven days.

Methods of stem cells preparation

Sampling

Bone marrow stem cells: The posterior iliac spine was sterilized, and local anesthesia, 1% Xylocaine, was applied to the periosteum and

the skin of that area. The bone marrow was then aspirated (100 mL), from each side, under aseptic conditions on preservative-free heparin.

Umbilical cord stem cells: Cord blood was collected ex-utero in Caesarean sections. Cord blood was collected after informed consent of mothers in Ghamra Military Hospital, and subjected to complete infectious disease investigations.

Mesenchymal stem cells (MSC) separation

Mesenchymal stem cells were separated using standard double physical separation. Briefly, mononuclear cells (MNC) separation was done using density gradient (Ficoll-Plaque 1.077 g/mL, GE Biosciences, Piscataway, NJ, USA). MNCs were washed with Dulbecco's low glucose modified Eagle's medium (Hyclone, Logan, UT, USA) containing 1% antibiotic-antimycotic (Gibco, Grand Island, NY, USA), resuspended in complete culture medium (Minimum Essential Media [MEM], Gibco/BRL, Gaithersburg, MD, USA); 200 ul

of the patient's own serum, 100 units/ml of penicillin/streptomycin, and 2 mM L-glutamine (Gibco/BRL). Cells were plated in T25 flasks (Falcon Plastics, Los Angeles, CA, USA) at a density of 500,000 cells and incubated at 37°C, with 5% carbon dioxide. After 24 hours, non-adherent cells were discarded, and the adherent cells were morphologically evaluated every other day with media change until

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One month post-operative



Five months post-operative

Figure 6. Photos of patients in the BM-MSc group.



Pre-operative photos



Figure 7. Photos of patients with bone marrow stem cells application.

80-90% confluence is reached.

Harvest: adherent cells were then washed with phosphate-buffered saline (PBS) and harvested by incubation for 4 minutes at 37°C in 4 mL of 0.25% trypsin-EDTA. After incubation, the trypsin was inactivated by the addition of 5 mL of MEM. The cells were washed twice with PBS.

Cell viability was determined using the trypan blue exclusion test. According to the International Society for Cellular Therapy position statement in 2006, mesenchymal lineage was confirmed by plastic adherence, fibroblast-like morphology, trilineage differentiation potential and typical immunophenotyping [14].

Progress assessment

General Assessment: vital sign (pulse, temperature, blood pressure and urine output); Laboratory data (serum albumin level, hemoglobin percent, renal and liver functions) every three days.

Local Assessment: assessment of the status of the wound bed and granulation tissue.

Study groups were compared regarding: Rate of healing; Occurrence of early complications: a. Graft loss; Infections; Occurrence of late complications: a. Hypertrophic scars; b. Keloid; c. Contracture; d. Hypopigmentation or hyperpigmentation; Assessment of hospital stay and cost of treatment.

Follow up of the patients, after discharge; two weeks, one month, three months, and six

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Figure 8. Photos of patients with bone marrow stem cells application.

months for studying the post burn wound healing.

Statistical analysis

All results in the present study were expressed as mean \pm SD of the mean. Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) program, version 20.0 was used. Differences were considered significant when $P \leq 0.05$. Frequency and percentage of non-numerical data were performed.

Results

Demographic data of the studied patient groups are summarized in **Table 1** and **Figure 1**.

Rate of healing

Regarding the extent of burn, initial percentage ranged between (15-25%), (12-22%) and (10-

20%) in the three groups respectively. After follow-up, the percentage of burn extent was significantly reduced in both UCBS and BMSC groups as compared to early excision and graft group; with insignificant difference between UCBS and BMSC groups.

Hospital stay

The period of hospitalization was significantly reduced in both bone marrow stem cells group and umbilical cord blood stem cells group when each group was compared to early excision and graft group. Non-significant changes were observed when comparing bone marrow stem cell group to umbilical cord blood stem cells group as shown in **Table 1** and **Figure 1**.

Early complications

Regarding early complications which include infection, partial and total loss of graft as shown in **Tables 2-5** and **Figure 2**, the control (EE&G) group did not manifest any

early complications in 50% of cases while the other 50% of cases in this group developed either graft loss (alone in 10% of cases, combined with infection in 15% of cases) or infection (25% cases). In BM-MS group **Figures 5-8**, there was no early complication (in 75% of cases), while infection was present as an early complications (in 25% of cases). In UC-MS group **Figures 9-11**, 30% of cases did not have any manifestations of early complications while 70% of cases were complicated with infection.

Late complications

Data represented in **Table 6** showed the using of BMSCs in treatment of patients resulting no late complication 55% versus (5% and 30%) for the EE&G and UCMSCs respectively. While patients treated with UCMSCs showed hypopigmentation 10% versus (20% and 20%) for EE&G and BM-MSCs respectively. Patients

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Figure 9. Photos of patients in the UCMSC group



Figure 10. Photos of patients in the UC-MSC group

treated with BMSCs exhibit no hyperpigmentation versus (20% and 30%) for the group EE&G and UCMSCs. Group treated with BM-MSCs and UCMSCs exhibits 10% of contracted scar when compared with EE&G group. However, lower values in groups treated with stem cells either BM-MSCs or UCMSCs (15% and 20%) versus 40% for EE&G group. These differences were statistically signed as shown in **Table 6** and **Figure 2**.

Treatment expenses

The total cost of hospitalization of patients admitted in El Helmia Military Burn Center (admission and treatment) was estimated in Egyptian currency. The patients in excision and graft group costed maximum 20.500 thousand Egyptian pounds versus 20-21 thousand for the other groups. The cost also ranged between (11-20.5), (15-20) and (15-21) in the groups respectively as shown in **Table 1** and **Figure 1**.

Discussion

Major burns represent a considerable health problem, with dual burden both on individuals and on the health system. Patients suffering from handicapping wounds require frequent operations, prolonged hospitalization and intense rehabilitation [15].

Standard management of major burn patients consists of autologous skin grafting followed by application of an elastic bandage. However, donor sites are limited especially in extensive deep burns *Lu et al.* [3]. Efficient wound healing requires full thickness regeneration of the skin and its appendages; a target which is rarely achieved in conventional management, and

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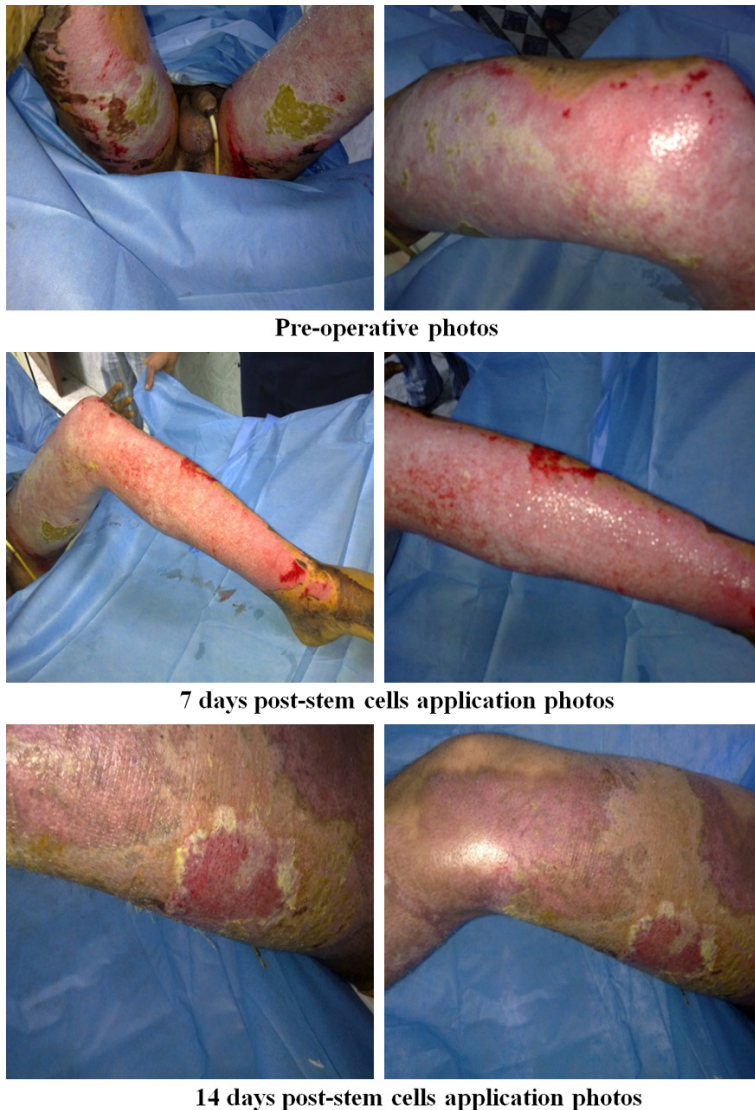


Figure 11. Photos of patients in the UCMSC group

which the application of stem cells for burn wound healing can achieve.

The use of stem cells in burn management as an alternative to EE&G could be beneficial, not only to temporarily prevent the loss of body fluids and bacterial infection, but also to achieve satisfactory repairing effects [16, 17]. Also, it was reported that stem cell application in the burn either by local spray with umbilical cord blood derived stem cells or by injection of bone marrow stem cells deep the burned area after excision were applied as a new method for regeneration of skin and decrease complication of skin graft or donor site [3, 18].

This study recruited patients with recent deep burn of up to 25%; randomly divided into three groups: conventional treatment (early excision and grafting EE&G), bone marrow derived mesenchymal stem cells (BM-MSC) and umbilical cord derived mesenchymal stem cells (UCMSC). Patients and controls were followed-up to evaluate wound healing, incidence of early and late complications as well as duration and cost of hospitalization.

In this study, evaluation of the rate of healing indirectly through the duration of hospital stay showed significant decrease in both stem cell groups as compared to the control group; with no significant difference between the bone marrow and cord blood derived mesenchymal stem cell groups.

In the current study, 50% of the control group (EE&G) developed either early complications, such as infection and graft loss, or late complications, such as keloid, hypertrophic scars and limitation of movement. On the other hand, early complications occurred in less incidence, in both

BM-MSC and UC-MSC groups as compared to the conventional EE&G.

The results encountered in the present study revealed that hypertrophic scar and limitation of movement were observed in early excision and graft patients, which served as a control group because it is the usual procedure in 10-25% TBSA burn.

Mohammadi et al. [19] studied the difference between early and late excision and graft in 50 patients suffering from deep thermal burn 30% TBSA. No statistically significant differences were found between both groups either

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Table 6. Comparison of late complications in the different studied groups

	Early Excision and Graft group		Bone Marrow Stem Cells group		Umbilical Cord Blood Stem Cells group		Chi-square test	
	No.	%	No.	%	No.	%	X ²	P-value
No complication	1	5%	11	55%	6	30%	17.819	0.022
Hypopigmentation	4	20%	4	20%	2	10%		
Hyperpigmentation	4	20%	0	0%	6	30%		
Contractured scar	3	15%	2	10%	2	10%		
Hypertrophic scar	8	40%	3	15%	4	20%		

in deformity, scar formation, sensation, development of disability as well as overall satisfaction. The most common site of involvement was the metacarpophalangeal (MCP) joint with frequency of 39% and 40% in early and late excision groups.

As regards duration of hospitalization, there was significant decrease in both bone marrow stem cells group and umbilical cord blood stem cells group when each group was compared to early excision and graft group. Non-significant changes were observed when comparing bone marrow stem cell group to umbilical cord blood stem cells group.

The explanation of the increase the time of hospitalization in the 1st group (ES&G) is the presence of early complication in a 50% of patients in this group as infection and partial loss of the graft.

Regarding the direct cost of hospitalization, non-significant changes were observed between the three groups regarding the direct cost of treatment. However, the overall cost of treatment may decrease as a result of decrease the time of hospitalization.

Zou et al. [20] found positive experience of using topical applications of non-processed autologous bone marrow aspirates on non-healing chronic wounds. Similarly, the applied autologous bone marrow aspirates to chronic unhealed burn wounds and slow-healing donor site wounds and there was a dramatic healing response observed in the present study.

Branski et al. [21] studied umbilical cord blood stem cells application by using a fibrin matrix after seeding stem cells, however, a Gentamycin cream was used in the present study

with Vaslin guse which was effective against local bacterial infection.

Huang and Burd [22] suggested that stem cells can be used even in patients scheduled for autologous skin grafting to improve the vascularity of the wound bed before operation.

Yamaguchi et al. [23] explained that MSCs have a tremendous ability for differentiation into both hematopoietic cells including endothelial cells, osteoblasts, neural, fibroblasts and keratinocytes. MSCs transdifferentiated into endothelial progenitor cells (EPCS) play an important role in neovascularization and wound healing.

Also, *Ravari et al.* [24] studied the effect of bone marrow MSCs in both acute and chronic wounds including deep burns. They reported that the combined therapy using topical application and edge injection of the bone marrow aspirates together with application of culture-expanded BM-MSCs resulted in successful healing of the previously non-healing ulcers and in treating the radiation burns. Both local (topical or subcutaneous injection) and systemic applications have been used. With respect to the local application, the concomitant use of acellular matrices or scaffolds was shown to increase cell homing, differentiation, mobilization and adhesion [17, 18]. In the present study, in the group of umbilical cord blood stem cells the used application of stem cells was applied either local injection or spray but without scaffold application.

Balaji et al. [25] explained stem cell-based therapy for wound healing in epithelium lined organs. Immediately after injury, there is a surge in the inflammatory response at the wound site to eliminate microbes and activate the healing process. MSCs interact with adjacent epithelial and inflammatory cells, initiating

a combination of cellular and paracrine effects promoting efficient wound healing. Stem cells applied to wounds can contribute to healing by numerous effects, namely, direct transdifferentiation into the required cell types, secretion of growth factors and effector molecules integrating the regeneration processes, stimulation of proliferation of resident stem cells and lastly stimulation of angiogenesis needed for tissue regeneration.

In conclusion, the use of MSCs to enhance wound regeneration in deep burns represents a new modality offering great hope for this patient group.

Declaration of conflict of interest

None.

Address correspondence to: Hala Gabr, Department of Clinical Pathology, Cairo University, Cairo, Egypt. E-mail: halagabr@yahoo.com

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