Research Article

Evaluation of the Effect of Cell Spray Technique in the Treatment of Partial-thickness Skin Loss.

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Abstract

Introduction: Early excision and prompt resurfacing with skin grafts is the mainstay of surgical treatment of extensive raw areas. However, early excision with autograft coverage may be difficult to be achieved in patients with extensive burns due to the limited size of donor sites. **Aim of the work:** The aim of this study is to evaluate the treatment of post-burn and post-traumatic raw areas using Cell Spray Technique to better understand its indication at a maximum benefit. **Patients and methods:** 20 Patients aged from 2-40 years old with a partial-thickness to deep partial-thickness wound of up to 20% total body surface area (TBSA) requiring surgical debridement and skin grafting participated in this study, underwent cell spray-on grafting on their raw are after harvesting a normal graft from their donor site, separate dermis from epidermis, mince the epidermis and put it in a suspension solution of ringer Lactate. **Results:** Majority of patient rejected the sprayed cells or showed subsequent scars that scored high on the Vancouver Scar Score. **Discussion:** We tried to apply the same clinical technique of the ReCell device in our study, to best achieve the benefits of minimizing the donor site to cover up larger recipient defects in an economic way, by substituting every tool included in the ReCell device kit with a cheaper tool from our operating field, trying to maintain the level of quality of the healing process and the resultant scar outcome. We tried to assess the quality of healing process and the resulting scar, and its relation to every factor we managed in the criteria and to figure out how far these elements affected the results of our study. **We recommend:** Further trials of the technique to achieve higher healing quality, and thorough selection of the candidate patients to avoid factors that disrupt the healing process and worsen the resultant scar.

Keywords: Cell spray, CEA, Re Cell, autologous cell harvest, graft, raw area.

Introduction

Early excision and prompt resurfacing with skin grafts is the mainstay of surgical treatment of extensive raw areas[(1).] Studies of these treatments have clearly shown early excision and grafting will improve survival, reduce hypertrophic scarring, decrease pain duration, shorten hospital stay and reduce infectious complications. With this principle in mind; a wide variety of techniques and methods have been applied for coverage of an open post-burn or post-traumatic raw area, including meshed and non-meshed autografts, homografts or a combination of both, human amniotic membrane, xenograft, synthetic skin substitutes, and cultured epithelial autografts (CEA)[(2).]

However, early excision with autograft coverage may be difficult to be achieved in patients with extensive burns due to the limited size of donor sites. Definitive coverage of all raw areas may take several weeks while waiting for donor sites to epithelialize for repeated harvesting or for CEA to grow. Delays may also be due to systemic illness or local wound infection that can develop while waiting for CEA. These delays in autograft coverage have led to the development of a noncultured epithelial autograft which enables the surgeon to greatly expand the amount of coverage they can obtain from small donor sites with immediate application[(3).] Additionally; early difficulty with transferring CEA to the wound beds lead to the development of a spray-on technique, which allows for easy transfer of proliferating keratinocytes to the wound [(4).]

An autologous cell harvesting (ACH) system (ReCell, Cell Spray) enables the surgeon to immediately process a small split-thickness biopsy and deliver keratinocytes, melanocytes, fibroblasts, and Langerhans cells harvested

from the epidermal-dermal junction to the wound bed using a spray-on technique. This technique can cover up to 80 times the area of the split-thickness skin biopsy[5].

Some authors claim it is as efficient as a skin graft with the added advantage of a significantly smaller donor site. Its use with skin substitutes and several wound dressings have also been shown to be safe. Despite these potential benefits, NICE (National Institute for Clinical Excellence) evaluation has not defined the indications or use of Cell Spray in raw areas management. Its use varies between burn units and appears to depend on surgeon preference [6].

Aim of the work

The aim of this study is to evaluate the treatment of post-burn and post-traumatic raw areas using Cell Spray Technique to better understand its indication at a maximum benefit.

The goal is to achieve a wound healing by a recent approach to transfer the skin cells from the donor site to the recipient defect as closely as possible and to reduce donor site morbidity.

Patients and methods:

Patients:

Patients with a partial-thickness to deep partial-thickness wound of up to 20% total body surface area (TBSA) requiring surgical debridement and skin grafting were asked to participate in this study, considering the following criteria:

Inclusion criteria

- Both sexes were included.
- Children and Adults between 2-40 years of age.
- Patients with a less than 20% TBSA partial-thickness to deep partial-thickness injury requiring skin grafting.

Exclusion criteria

- Patients below 2 and above 40 years.
- Patients with more than 20% TBSA partial thickness skin loss.
- Presence of a microbiologically proven pre-existing local or systemic bacterial infection.
- Taking medications known to influence wound healing or skin pigmentation (e.g. steroids or cytotoxic drugs).
- Presence of a pre-existing condition that may interfere with wound healing (e.g. malignancy, diabetes or autoimmune disease)
- Known hypersensitivity to trypsin or ringer lactate solution is present.
- Refusal to be enrolled in the study.

Pre-operative management:

20 Patients among those who met all inclusion and exclusion criteria were enrolled into the study. Patients were informed about the potential risks and benefits of the procedure and a signed consent was obtained from each of them.

A careful medical history, thorough physical examination including: CNS, chest, heart, abdomen, lower limbs and back and necessary investigations was done.
Candidate patients were subjected to either one of the followings according to wound contamination degree:

- ***In case of post-burn***: debridement and tangential wound excision or escharotomy was done under general or local anesthesia according to the size of the wound, then topical antibiotics and silver sulfadiazine was applied with daily dressings for 5 days to a week before the procedure was carried out.
- ***In case of post-traumatic***: topical antibiotic was applied and quick mechanical wound debridement was done on the spot before the procedure was carried out.

On our initial evaluation, the patients were diagnosed to have either partial-thickness or deep partial-thickness skin loss, and thus considered a candidate for single-cell spray application as an alternative to STSG.

Methods:

Equipment used the procedure

1. Alpha-chemotrypsin ampoules for separation of the graft.
2. Ringer's lactate solution for suspension.
3. Hot saline bath for rising the temperature of the separation media.
4. 10 ml syringes for mixing the cells.
5. 20 ml syringes for spraying the cells.
6. Sterile plastic nozzle.
7. Sterile gloves.

8. Gauze and sterile wound dressing.

Procedure:
The entire procedure was performed in an operating room, ranging from 5 to 14 days post-burn/post-traumatic; the patient underwent single-cell transplantation. We harvested the graft from right or left hip as a donor site. The graft was taken to a depth of 0.008 in using a Waston Skin Graft knife after preparation either under general anesthesia or sub-cutaneous 2% lidocaine with epinephrine was given.
The tissue was procured into a single cell suspension for cell spray transplantation. The cell isolation procedure was performed on site in the same room immediately prior to cell transplantation.

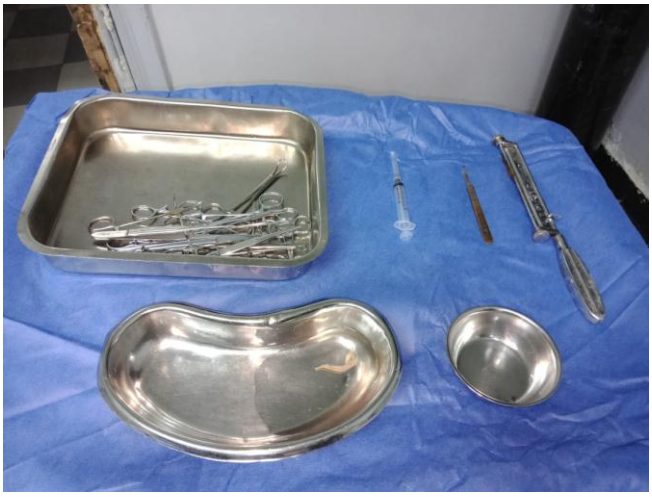

Fig (1): Equipment used in the procedure

Surgical technique:
After skin harvest from donor site, the tissue was handled under totally sterile conditions until application and maintained under the direct supervision of the surgeon. During donor skin processing and before application, the wound was prepared by tangential excision and gentle mechanical debridement until bleeding spots appeared. Hemostasis was achieved by regular gauze.

The harvested graft was subjected to enzyme approach of tissue digestion using alpha-chemotrypsin enzyme, and cell washing using Ringer's lactate solution. The steps of autologous skin cell spray transplantation required approximately 75 minutes.

The donor site graft was harvested in a routine way, as known for split skin grafting. Initial separation of dermis and epidermis for 25-40 min was done in a solution of 15 ml alpha-chemotrypsin (2 ampoules) and 5 ml of Ringer's lactate at 37 C in a sterile container.

Mechanical separation of dermis and epidermis using forceps and scalpel was performed in a dry container (the specimen must not dry out), the dermis parts are not used further, transferring the epidermal graft in a sterile tube with Ringer's lactate solution, to be washed from the enzyme.

The epidermal graft was then transferred into a new sterile container and cut into 3-4 mm pieces with a surgical disposable scalpel, and these mini epidermal pieces were minced with the scalpel to form a homogenous cellular paste. We took care that the specimen wouldn't dry out.

The epidermal paste was placed into a single cell suspension for 10-15 min in a 5 ml Ringer's lactate solution at 37^{o}C, to allow isolation from

epidermis, then sieving the cell suspension through a 70 micrometer proline surgical mesh used as a sieve substitute happened.

The sieved cells were put into clinical grade Ringer's lactate solution, and then transferred into 20 ml sterile disposable syringes for cell deposition by spraying. One syringe at a time, the cell spraying was performed either by using sterile plastic nozzle applied to the syringe, or by dripping the suspension out of the syringe directly on the wound bed, through which the suspended cells were immediately spray-transplanted onto the wound of the patient by spray deposition.

Results

All the patients subjected to follow up visits on the weeks 1, 2, 3, 4 and 6 postoperative for dressings and monitoring the healing process. After which all the resultant scars were be assessed using Vancouver Scar Score to assess pigmentation, vascularity, pliability and height. Our results recorded that the patient characteristics, wound status, duration between injury and procedure and other factors were comparable between the enrolled patients.

Table (1): Relation between (Special habits & Medical history) with Vancouver Scar Scores

	All cases	**Vancouver Scar Scale (VSS)**				**P value**	**Statistically significant**
		0 to 4	5 to 8	9 to 13	Fail		
Total	20	2	6	7	5		
Special habits							
Non-smoker	13	2	4	6	1	0.0277	Sig.
Smoker	6	0	2	0	4		
Smoker/Alcohol	1	0	0	1	0		
Medical history							
None	16	2	6	4	4	0.5623	N.S
HTN	3	0	0	2	1		
HTN & Cardiac	1	0	0	1	0		
Statistical test used: Chi-Square test							
p-value≤0.05 considered statistically significant (95% confidence interval).							

These results suggest that cell spray technique provides a method for the preparation of a cell suspension with high viability and proliferative potential, but this technique have more than one factor affecting the healing process and quality of the resultant scar, first factor was age, especially between (11-23) years, young patients' transplanted cells had more vulnerability to slough and shed due to sheering movement and lesser control on mobility in the early postoperative days, fragility of the sprayed transplanted cells could not resist in such patients leading to failure to heal.

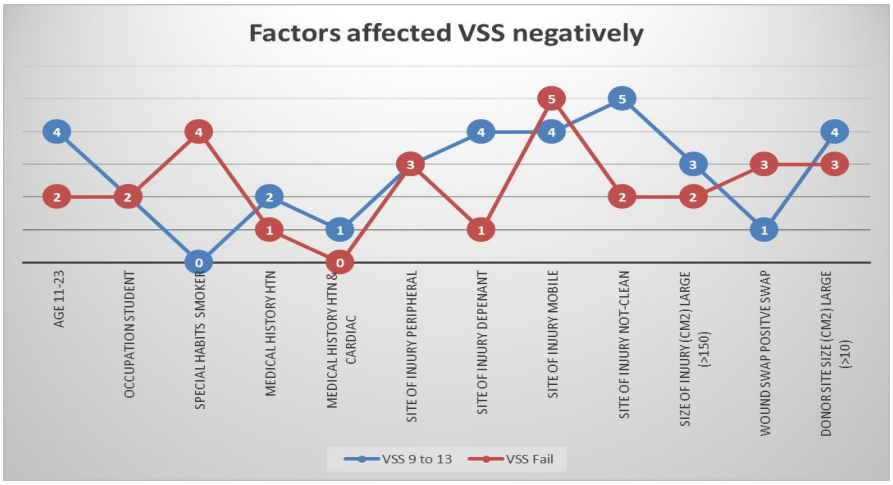

Fig (2): Factors affected Vancouver Scar Scores negatively

Other factors like medical history, site of injury (peripheral/dependent/mobile/not clean), preoperative infection, and donor site size were enough reasons to worsen the subsequent scar after healing, but the most effective factors found, which must be highly considered in the future plans using this technique were smoking and large size of injury. Smoker patients with large size of injury had the greatest tendencies to form an irregular, less elastic, hypo or hyper pigmented scar, and scored the highest numbers on Vancouver Scar Scale.

Table (2): Logistic Regression Test

Factors	Odd Ratio	P-Value
Age	1.019	0.710
Sex	1.519	0.702
Smoking	17.909	0.031
Medical history	0.517	0.667
Size of injury (cm2)	1.009	0.118
Cause of injury	0.981	0.978
Duration between onset of injury and procedure	1.336	0.633
Wound swap	2.921	0.113
Type of organism	2.549	0.131
Donor site size (cm2)	0.545	0.576

Discussion

Skin wounds represent a major healthcare problem owing to an increasing number of trauma and pathophysiological conditions. Normal wound healing process includes a very well-orchestrated and regulated process consisting of series of events such as haemostasis, inflammation, proliferation and ECM remodeling [7].

The gold standard for burns is early excision followed by wound coverage. This is usually achieved using autologous split thickness skin grafts (STSG), harvested elsewhere on the not burned part of the body of the patient. However, the limited number of donor sites in severely burned patients and the risk of contraction, due to the lack of dermis, remain the biggest

obstacles. Skin expansion techniques (e.g. mesh, meek) result in even more scarring [8].

One of the most important milestones in the history of burn care is the introduction of skin grafting (STSG and FTSG) initially described by **Reverdin in 1869**. He observed epithelial islands during the healing process of burned skin; confluence of those epithelial islands resulted in accelerated wound healing, even when these islands were artificially grafted on the granulating tissue. Numerous clinical experiments showed that healing is more rapid in a clean wound bed and that the thickness of the graft is important (the thinner the graft, the faster wound closure was realized). Complete coverage of the wound with grafts seemed to result in better scar quality, suggesting the need for larger grafts [9].

In 1964, **Tanner** unleashed a revolution by developing the technique of meshing a skin graft: a simple procedure to expand the autograft to bigger surface. Combination of meshed grafts with cadaveric skin, the so called "Sandwich technique" first advocated by **Alexander** in 1981, became the standard treatment of severe burn patients. However, expansion techniques resulted in more visible scarring because of the slower healing and persisting grid image caused by contraction. Moreover, the meshing technique cannot deal with the shortage of donor sites in the most extensive burns [10].

The concept "Tissue Engineering" has been defined in 1987 by the US National Science Foundation. This concept comprises Keratinocytes in the treatment of severe burn injury the construction of new tissue by combining cells with a scaffold and growth factors [11].

Nowadays research focuses on further improvement of culturing techniques and the combination of keratinocytes with a scaffold to form skin equivalents.

In spite of the development of different types of skin substitutes (ie, Epicel and Apligraf) for the clinical setting, application of skin substitutes face some limitation such as high cost, low efficiency, and inability in complete reconstitution of skin appendages.117 Besides the cultivation/manufacturing process, there is some inherent limitation of cultured epithelial autograft (CEA) sheets for treatment of skin disorders, such as storage and handling of fragile CEA [12]. Moreover, in cases of clinical applications of CEA sheets, one serious limitation is that it takes a long time to reach the multilayered complete CEA sheets that are suitable for grafting.

In 2011, Gerlach et al., in contrast to Wood et al., employed a modified two enzyme isolation technique involving dispase and trypsin, together with cell washing by centrifugation. While cell washing removes remaining enzymes prior to grafting, the application of dispase enabled to separate dermis from epidermis and this allowed the subsequently used trypsin to isolate the cells from the epidermis-dermis interface, specifically the otherwise enclosed layer of the epidermis that contains regenerative basal keratinocytes. These skin progenitors are not selectively reached by the conventional technique. They reported how their modified method and clinical implementation in an outpatient setting for a patient that exhibited delayed wound healing after conservative therapy of a moderate deep partial thickness burn wound went, resulting in complete re-epithelialization of the dry wound with excellent both functional and cosmetic results [13].

They also assumed that the seeded cell density in the burned area will determine the speed of healing process and the spray pattern will determinate efficiency covering the wounded surface. Higher cell density would provide more cells in the wound, increasing the number of cell divisions and, in turn, would accelerate the wound healing process. However, higher cell densities would also require larger skin donor sample areas, making the technique less advantageous than regular skin mesh graft. Seeding keratinocytes at a density of 104 cells/cm2 increases 10-fold the cell expansion, on average, covering the entirety of the burned surface [14].

On September 2018, AVITA Medical (a clinical and commercial company developing

and marketing a range of respiratory and regenerative products) launched The ReCell® Spray-On Skin system in the US market, the device that made a revolution in the skin grafting field for partial-thickness burnt patients, after approval of FDA (Food and Drug Association) on February 2018.

The ReCell Autologous Cell Harvesting Device is is noncultured, meaning that it avoids the long laboratory time - typically a number of weeks - required to culture skin autografts, which are skin grafts grown from an individual's own skin cells. It is also autologous, meaning that it uses the patient's own cells and thus avoids triggering an immune reaction or risking an immune rejection.

Conclusively, ReCell Spray-On Skin is an autologous cell harvest and delivery system that does not require culture time. Instead, a small split-thickness biopsy is obtained during surgery and digested with trypsin. The epidermis is then separated from the dermis and the cells are disassociated and suspended in Lactate solution resulting in a cell suspension consisting of predominantly keratinocytes (> 60%), but also containing fibroblasts and melanocytes .This cell suspension is then applied to the prepared wound bed within an hour of isolation.

ReCell® differs from CEA, in that it is a non-cultured epidermal/dermal autograft. ReCell® has added the advantage of completing the dermabrasion/autograft surgery in a single procedure with a minimal donor site, leading to shortened time of operation. In the standard method of application for CEA, a punch biopsy site can take up to 3–4 weeks of preparation before grafting, while the whole procedure with ReCell can be performed in approximately 1 hour [(15)].

Given that the price of ReCell kit is very expensive and not affordable by most of the patients, in addition to its unavailability in the Egyptian and the Middle Eastern market. We will try to apply the same clinical technique of the device in our study, to best achieve the benefits of minimizing the donor site to cover up larger recipient defects in an economic way, by substituting every tool included in the ReCell device kit with a cheaper tool from our operating field, trying to maintain the level of quality of the healing process and the resultant scar outcome.

We tried to assess the quality of healing process and the resulting scar, and its relation to every factor we managed in the criteria and to figure out how far these elements affected the results of our study.

Summary and conclusion

This study aimed to evaluate the efficacy of application of Cultured Epidermal Autograft (CEA), in the form of spray cell suspension. In order to accelerate the wound healing process, minimize the donor site and decrease its morbidity, maximize the recipient defect benefitting from the graft procedure and achieve better scarring results, all in an economic affordable technique using cheaper tools and materials.

The patients were allocated into 4 groups according to assessment of the resultant scar by more than one person (including Senior Authors) with Vancouver Scar Score:

1. **Group 1:** 5 patients whose wounds failed to heal and thus were out of assessment by Vancouver Scar Scale.
2. **Group 2:** 7 patients developed scars that scored from 9 to 13 on Vancouver Scar Scale.
3. **Group 3:** 6 patients developed scars that scored from 5 to 8 on Vancouver Scar Scale.
4. **Group 4:** 2 patients developed scars that scored from 0 to 4 on Vancouver Scar Scale.

All patients were assessed in terms of:

1. Sex, age, occupation, medical history and special habits of medical impor-tance: analyzing how these factors affected wound healing and cells acceptance.
2. Site and size of the injured area, size of donor site and how this implemented success of the procedure.
3. Duration between onset of injury and carry out of the procedure.
4. Wound contamination and suspected infection: wound swab was done to all the patients after 2 days of sterile dressings

with topical antibiotics. Accordingly, patients with positive results were given systemic antibiotics specific to the organism detected in the swab result, and topical specific antibiotic was applied for 5 days to a week before carrying out the cell-spraying transplantation procedure.

Our results recorded that the patient characteristics, wound status, duration between injury and procedure and other factors were comparable between the enrolled patients.

These results suggest that cell spray technique provides a method for the preparation of a cell suspension with high viability and proliferative potential, but this technique have more than one factor affecting the healing process and quality of the resultant scar, first factor was age, especially between (11-23) years, young patients' transplanted cells had more vulnerability to slough and shed due to sheering movement and lesser control on mobility in the early postoperative days, fragility of the sprayed transplanted cells could not resist in such patients leading to failure to heal.

Other factors like medical history, site of injury (peripheral/dependent/mobile/not clean), preoperative infection, and donor site size were enough reasons to worsen the subsequent scar after healing, but the most effective factors found, which must be highly considered in the future plans using this technique were smoking and large size of injury. Smoker patients with large size of injury had the greatest tendencies to form an irregular, less elastic, hypo or hyper pigmented scar, and scored the highest numbers on Vancouver Scar Scale.

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